Guidance on Nanomaterial Hazards and Risks

Final Report of Work Completed in Phase I of an

Interagency Agreement between the National Institute for Occupational Safety and Health and the U.S. Army Center for Environmental Health Research

May 21, 2015

NIOSH Nanotechnology Research Center

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Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
21 MAY 2015		

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Guidance on Nanomaterial Hazards and Risks	**IAA # 9955473**
	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
NIOSH Nanotechnology Research Center	5e. TASK NUMBER **X1**
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
U.S.Army Center for Environmental Health Research,568 Doughten Drive,Fort Detrick,MD,21702-5010	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This report provides guidance from NIOSH to the Army on a research and data analysis strategy to provide the information needed to systematically evaluate the hazards, exposures, and potential health risks of engineered nanomaterials (ENMs) used in Army materiels. This information will provide support for risk management decision-making. NIOSH and the U.S. Army Public Health Command (PHC) have responsibilities for occupational safety and health related to nanomaterials. NIOSH focuses on workers and PHC focuses on occupational (i.e. soldiers and workers) and environmental risks (civilians and their families). At the present time, there are many uncertainties about hazards, exposures, and risks from nanomaterials. The rapid use of a relatively large number of nanomaterials by the U.S. Army requires a concerted effort to identify the processes and job tasks in which potential hazards may be present and exposures may occur so that appropriate risk management decisions can be made.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**		**101**	

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Overview

This report provides guidance from NIOSH to the Army on a research and data analysis strategy to provide the information needed to systematically evaluate the hazards, exposures, and potential health risks of engineered nanomaterials (ENMs) used in Army materiels. This information will provide support for risk management decision-making.

NIOSH and the U.S. Army Public Health Command (PHC) have responsibilities for occupational safety and health related to nanomaterials. NIOSH focuses on workers and PHC focuses on occupational (i.e. soldiers and workers) and environmental risks (civilians and their families). At the present time, there are many uncertainties about hazards, exposures, and risks from nanomaterials. The rapid use of a relatively large number of nanomaterials by the U.S. Army requires a concerted effort to identify the processes and job tasks in which potential hazards may be present and exposures may occur so that appropriate risk management decisions can be made.

This project has two main goals:

I. To suggest modifications to the overall health risk assessment process for Army materiel, as described in ASTM E2552-08, so that the process will be appropriate for the unique characteristics of nanomaterials. The scope for this effort includes health risks of nanomaterials in the Army throughout the product life-cycle (i.e., conception, synthesis, testing, demonstration, engineering and manufacturing development, storage and use, and demilitarization). Development of occupational exposure limits and other exposure values should be discussed. Additionally, research investigator safety should be considered.

II. To identify specific toxicity tests or other methods for evaluating potential human health effects of nanomaterials used in Army applications. Examples of issues to be addressed include appropriate nanomaterial characterization, dose metrics, the ability to extrapolate to specific health effects, human health studies, and the applicability of structure-activity relationships. The guidance will show where the recommended testing fits within the staged development of Army materiel (e.g., *in vitro* screening tests early in development; additional, less uncertain, yet more expensive and time-consuming *in vivo* testing only at later stages of development).

To reach these goals, NIOSH is developing guidance for how to develop information concerning hazard, exposure, and potential health effects related to nanomaterials that the U.S. Army is currently using and for new ones that they might use in the future. Essentially, this guidance is based on an approach that involves nanomaterial characterization, placing the nanoparticle in a mode of action category, and decision logic that prescribes approaches to tiered toxicity testing (involving *in vitro* and *in vivo* tests) and methods for risk assessment, including health effects, which would lead to health-based or provisional occupational exposure limits. Specifically, NIOSH is developing a comprehensive strategy for obtaining necessary information in which to make risk management decisions for soldiers and workers exposed to nanomaterials. The tasks in Goal II include:

1. Review of the list of nanomaterials identified by the army; from their physico-chemical properties place nanoparticles into mode of action and fate/transport categories for toxicology testing; suggest positive and negative particles for these categories. Discuss additional characteristics (e.g. agglomeration) that may help in the categorizing and ranking for testing.

2. Describe a tier strategy for toxicity testing; identify appropriate *in vitro* tests for each mode of action category. Testing scheme would allow a potency ranking for each member of a category using in vitro screening, verify selected members in this potency sequence using bolus in vivo testing, verify the bolus results with selective inhalation testing. The potency of members of a category could be compared to the potency of known positive and negative control particles to give relative hazard.

3. Suggest methods to evaluate dustiness of nanoparticles.

4. Suggest methods to evaluate worker exposure (particle size distribution, count, mass, etc.) during processing and incorporation into materials and soldier exposure in the field.

5. Describe the current hazard and control banding options for nanomaterials, and develop initial exposure bands based on available data.

6. Describe controls to be employed to reduce exposure for a given hazard band.

7. Describe methods to evaluate exposure-health effect relationships for selected nanomaterials.

Acknowledgments

The report was developed by scientists and staff of the National Institute for Occupational Safety and Health (NIOSH) who participate in the NIOSH Nanotechnology Research Center (NTRC). Charles Geraci, PhD, CIH, is the Associate Director for Nanotechnology. The document writing team included Eileen Kuempel, PhD, Jenny Roberts, PhD, Adrienne Eastlake, MS, Aleks Stefaniak, PhD, and Ralph Zumwalde, MS. This report describes work completed under an Interagency Agreement (IAA # 9955473) between the National Institute for Occupational Safety and Health and the U.S. Army Center for Environmental Health Research.

Special thanks goes to MAJ Jonathan D. Stallings, PhD, MAJ, MS, Director, Environmental Health Program, US Army Center for Environmental Health Research (USCEHR), Mark Widder, USCEHR, and William H. van der Schalie, PhD (formerly with USACEHR) for their support, review, and contributions to the IAA. Comments from Mark Hoover, PhD, Division of Respiratory Disease Studies (DRDS) are also appreciated.

Project Goal I. Suggest modifications to the overall health risk assessment process for Army materiel, as described in ASTM E2552-08

The ASTM guidelines are generally consistent and complementary to the NIOSH suggested framework for assessing the potential occupational health risks of engineered nanomaterials (ENMs). Suggestions to enhance and refine these guidelines are provided in this report, and as discussed below. Additional suggestions may be provided as further NIOSH guidance is developed.

Sections 4.1 and 5.3: In addition to the relative risk evaluations proposed, the inclusion of benchmark or reference particles in a tiered toxicology testing scheme would provide the opportunity for linking relative and absolute risk measures across ENM groups. This approach would facilitate the estimation of occupational exposure limits or bands (OELs or OEBs) for ENMs and provide information needed for the occupational safety and health decision-making, including the selection of engineering controls and personal protective equipment. More information is provided in Tasks 2, 6 and 7 of this report.

Section 5.1: Although the ASTM guideline for detailed exposure estimation at more advanced stages of development is reasonable (when the potential uses may be more clearly defined), it may also be feasible to develop initial exposure estimates at any stage of ENM research and development. This may include laboratory or pilot production facilities (e.g., for specific processes or uses of the ENMs). Additional suggestions are provided in Tasks 3 and 4.

Section 6.2.1 and 6.3.1: Quantitative structure activity relationships (QSAR) modeling would be an ideal approach to evaluate the potential hazard and risk of ENMs. However, the list of general properties in these sections would need to be refined and extended to include those most relevant for evaluating bioactivity and toxicity. The tiered toxicology testing framework based on biological mode of action (MOA) and associated physico-chemical (PC) properties suggested by NIOSH (in Tasks 2 and 7) would facilitate the development of a robust data base for QSAR modeling.

Section 6.2.6: In addition to the ASTM examples of using data on the no observed adverse effect levels (NOAELs) or the acute doses associated with 50% lethality (LD50), the development of a database with a standard set of endpoints and dose measures in *in vitro* and limited *in vivo* experimental systems would facilitate the characterization of dose-response relationships across a set of ENMs. These assays should include sufficient dose groups to characterize the dose-response relationships at occupationally-relevant exposures, including relevant short-term or repeated exposure scenarios. This approach (discussed in Tasks 2 and 7) would facilitate the categorization of ENMs into health risk groups based on the biological MOA at exposures that may occur in during the production or use of the ENMs.

Sections 6.3.2 – 6.3.5: State-of-the-art suggestions for specific toxicity assays and measures are suggested in Task 2. These suggested assays provide a more fully developed framework for toxicity testing including evaluation of the scientific literature that has become available since the publication of the ASTM guidelines.

Section 6.4: A more comprehensive framework for tiered toxicology testing is suggested in Task 2. Like the ASTM guidelines, the NIOSH framework is efficient in using the lower tier testing data to prioritize ENMs for higher tier testing. In addition, the NIOSH framework would facilitate the development of hazard categories of ENMs through toxicological evaluations of ENMs based on MOA and PC properties. These assays would include benchmark or reference materials to facilitate comparative potency analyses and development of OELs or OEBs, as discussed in Tasks 5 and 7. The use of standard assays and endpoints, as discussed in Task 2, would help to reduce variability and uncertainty across methods and laboratories.

The toxicology testing and data analysis framework discussed in this report would build on the ASTM guidelines and facilitate the development of a robust database needed for hazard and risk assessment of ENMs and occupational health decision-making.

Project Objective II. Provide Guidance on specific toxicity tests or other methods to evaluate potential human health effects of nanomaterials used in Army applications

Task 1: Evaluate the list of ENMS in TEARR and select representative materials

Table 1 of ENMs in TEARR [Money et al. 2013] provides five nanomaterial categories: carbon-based, metals, metal oxides, inorganic, quantum dots (plus a sixth group: unknown). NIOSH has evaluated this grouping and provides a draft revision to Table 1 to group these ENMs to more closely align with the hazard and toxicology testing categories based on biological mode of action (MOA) and associated physico-chemical (PC) categories. These MOA-based categories are used to suggest specific toxicology testing assays for screening and prioritizing materials for higher tier testing (Task 2). Benchmark materials within each category are also suggested (Task 7), which serve as points of reference for comparison of hazard and dose-response data of well-characterized materials with the findings for the new nanomaterials evaluated in the same assays. These benchmark materials were also used as case studies for evaluation of current control banding strategies for ENMs (Task 5).

Table 1-1 below shows the current ENMs listing in TEARR. Table 1-2 provides a draft (preliminary) revised grouping by MOA for initial toxicology testing of materials within these groups. Specific ENMs within a general ENM class (e.g., clays, polymers) may belong in more than one group depending on the specific formulation, surface functionalization, or coatings. Further refinement of these groups is anticipated for materials with specific PC properties, or based on results of initial screening. For example, soluble materials may be subdivided into those for which the ion is associated with acute toxicity or those for which dissolution results in clearance and reduced toxicity with repeated exposure (compared to similar materials that are poorly-soluble and biopersistent). Fibrous (high aspect ratio) particles may require subcategories based on dissolution/biodurability. Other subcategories may be based on potency of response within the toxicology assays (e.g., associated with particle size or surface area). Thus, the hazard potential of

materials within a category need not be identical but may be related by a common mechanism that influences the dose-response relationship. This initial listing is based on evaluation of available information for similar materials [e.g., NIOSH 2007], the scientific literature of effects in *in vitro* and *in vivo* studies [e.g., Zhang et al. 2012; Liu et al. 2011, 2013; Cho et al. 2012; Rushton et al. 2010], and the Tool for ENM-Application Pair Risk Ranking (TEARR) database [Money et al. 2013].

Table 1-1. List of ENMs Included in TEARR [Money et al. 2013, Table 1].

Category	No.	EMN (Abbreviation in TEARR)
Carbon-based	1	Boron Carbide (B4C)
	2	Carbon Nanoparticles (Carbon)
	3	Carbon Aluminum Composite (CarbAl)
	4	Clays (Clays)
	5	Carbon Nanotubes (CNT)
	6	Fullerene (Fullerene)
	7	Graphene (Graphene)
	8	Graphite (Graphite)
	9	Misc (Misc)
	10	Multi-walled Carbon Nanotubes (MWCNT)
	11	Nylon (Nylon)
	12	Polymer (Polymer)
	13	Silica-coated Nanotubes (SiCNT)
Metals	14	Silver Nanoparticles (Ag)
	15	Aluminum Nanoparitlces (Au)
	16	Gold Nanoparticles (Au)
	17	Brass Nanoparticles (Brass)
	18	Cobalt Nanoparticles (Co)
	19	Copper Nanoparticles (Cu)
	20	Iron Nanoparticles (Fe)
	21	Germanium Nanoparticles (Ge)
	22	Lithium Aluminum Silicate Glass (LiAlSi)
	23	Nickel Nanoparticles (Ni)
	24	Palladium Nanoparticles (Pd)
	25	Platinum Nanoparticles (Pt)
	26	Silicon Nanoparticles (Si)
	27	Titanium Nanoparticles (Ti)
Metal Oxides	28	Alumina (Al2O3)
	29	Barium Titanate (BaTiO3)
	30	Cuprous Oxide (Cu2O)
	31	Cupric Oxide (CuO)
	32	Misc (Misc)
	33	Silica (SiO2)
	34	Titanium Dioxide (TiO2)
	35	Zinc Oxide (ZnO)
	36	Zirconia (ZrO2)
Inorganic	37	Tungsten Nanoparticles (W)
	38	Tungsten Disulfide (WS2)
	39	Ceramics (Ceramics)
	40	Cadmium Sulfide Quantum Dots (CdS)
	41	Cadmium Selenide Quantum Dots (CdSe)
	42	Cadmium Telluride (CdTe)
	43	Lead Sulfide Quantum Dots (PbS)
	44	Lead Selenide Quantum Dots (PdSe)
Unknown	45	Unknown

Table 1-2. Biological mode of action (MOA) grouping of ENMs Included in TEARR [Money et al. 2013, Table 1].

Initial MOA Category	Chemical category	No.	ENM (Abbreviation in TEARR)
Higher solubility particles – acute toxicity subcategory	Metals	18	Cobalt Nanoparticles (Co)
	Metals	19	Copper Nanoparticles (Cu)
	Metals	23	Nickel Nanoparticles (Ni)
	Metal oxides	30	Cuprous Oxide (Cu2O)
	Metal oxides	31	Cupric Oxide (CuO)
	Metal oxides	35	Zinc Oxide (ZnO)
Higher solubility particles – lower acute toxicity subcategory	Metals	14	Silver Nanoparticles (Ag)
	Metals	15	Aluminum Nanoparticles (Au)
	Metal oxides	28	Alumina (Al2O3)
Poorly soluble, lower toxicity (PSLT) particles	Carbon-based	1	Boron Carbide (B4C)
	Carbon-based	2	Carbon Nanoparticles (Carbon)
	Carbon-based	3	Carbon Aluminum Composite (CarbAl)
	Carbon-based	4	Clays (Clays)
	Carbon-based	6	Fullerene (Fullerene)
	Carbon-based	8	Graphite (Graphite)
	Carbon-based	12	Polymer (Polymer)*
	Metals	16	Gold Nanoparticles (Au)
	Metals	17	Brass Nanoparticles (Brass)*
	Metals	20	Iron Nanoparticles (Fe)
	Metals	26	Silicon Nanoparticles (Si)
	Metals	27	Titanium Nanoparticles (Ti)
	Metal oxides	29	Barium Titanate (BaTiO3)
	Metal oxides	34	Titanium Dioxide (TiO2)
	Metal oxides	36	Zirconia (ZrO2)
	Inorganics	37	Tungsten Nanoparticles (W)*
	Inorganics	38	Tungsten Disulfide (WS2)*
	Inorganics	39	Ceramics (Ceramics) *
Poorly soluble, higher toxicity (PSHT) particles	Carbon-based	7	Graphene (Graphene)*
	Metals	21	Germanium Nanoparticles (Ge)*
	Metals	22	Lithium Aluminum Silicate Glass (LiAlSi)*
	Metals	24	Palladium Nanoparticles (Pd)*
	Metals	25	Platinum Nanoparticles (Pt)*
	Metal oxides	33	Silica (SiO2) [crystalline]
	Inorganics	40	Cadmium Sulfide Quantum Dots (CdS)
	Inorganics	41	Cadmium Selenide Quantum Dots (CdSe)
	Inorganics	42	Cadmium Telluride (CdTe)
	Inorganics	43	Lead Sulfide Quantum Dots (PbS)
	Inorganics	44	Lead Selenide Quantum Dots (PdSe)
Fibrous (high aspect ratio) particles	Carbon-based	5	Carbon Nanotubes (CNT)
	Carbon-based	10	Multi-walled Carbon Nanotubes (MWCNT)
	Carbon-based	11	Nylon (Nylon)
	Carbon-based	13	Silica-coated Nanotubes (SiCNT)

NOTE: THIS IS A PRELIMINARY DRAFT GROUPING of MATERIALS *Indicates that further evaluation of physico-chemical properties (including solubility) and toxicological effects are needed to evaluate grouping for these materials.
Note: Of the 45 listed in Table 1 of Money et al. [2013], three "misc" or "unknown" materials were omitted.

This preliminary grouping of ENMs will be evaluated further in subsequent analyses (such as discussed in Task 7). In particular, the further development of the TEARR database to include specific toxicological endpoints would facilitate evaluation of the dose-response relationships for these ENMs or their "bulk" (micro-diameter) counterparts with similar chemical composition. These analyses would include clustering methods [e.g., Liu et al. 2011; Rallo et al. 2011] to identify relationships among the substances in the group and to evaluate specific grouping criteria. Extension of the TEARR database to include specific dose and response data from published toxicology studies will be key to evaluating the preliminary grouping in Table 1-2.

One of the key factors for determining a MOA-PC group is solubility. It is also a PC property which lacks standardized measurement methods and thus is a source of uncertainty in the grouping ENMs with respect to hazard given acute or chronic exposure. The International Standards Organization (ISO) is currently developing a document on the solubility of nanomaterials (Dr. Aleks Stefaniak is the NIOSH delegate to that ISO working group). The ISO document uses the term 'biodurability' to indicate that this property is only based on cell-free simulant experiments and does not account for *in vivo* clearance mechanisms. It provides a review of the numerous types of tests available for measuring biodurability in cell-free experiments (i.e., not accounting for *in vivo* clearance mechanisms), as well as existing international standards related to dissolution (although none are for inhalation exposure).

A number of factors influence dissolution *in vivo* including the exposure pathway. NIOSH is focusing on inhalation exposures in the Table 1-2 groupings. For example, some materials readily dissolve at near-neutral pH but do not dissolve (or very little dissolves) at acidic pH (and the opposite is also true). So, depending upon whether the particles are located in the lung airways or engulfed by alveolar macrophages, the same material may be highly soluble and/or poorly soluble. Zeta potential (reported in the TEARR database) may be indirectly relevant to solubility. Several studies demonstrate that nanoparticle zeta potential influences engulfment by cells, such that particles with high negative or positive zeta potentials are more readily internalized than particles with a zeta potential closer to zero. These important factors are not captured in cell-free experiments.

Another aspect to consider is the difference between chemical equilibrium solubility and biological solubility. In chemistry, solubility is based on how much of a material will dissolve into water until equilibrium is reached. In contrast, biological solubility is usually taken to mean dissolution clearance relative to mechanical clearance; thus, a highly soluble material clears predominantly by dissolution whereas a poorly soluble material clears predominantly by cell-mediated or mechanical mechanisms. In Table 1-2, the solubility categories may be more clearly defined biologically as "Particle solubility known to influence response – high acute toxicity" and "Particle solubility known to influence response – low acute toxicity." For example, the adverse biological effects of ZnO and CuO nanoparticles could be explained by their solubility and release of metal ions [Zhang et al. 2012]. Metal dissolution in that study was determined by inductively coupled plasma-mass spectrometry (Perkin-Elmer SCIEX Elan DRCII ICP-MS) [Ji et al. 2010]. Less soluble materials may generate reactive oxygen species (ROS) and cause toxicity due both to the reactivity of the nanoparticle surface and to the ion release during dissolution. Bio-solubility is a key parameter to measure experimentally (as discussed in Task 2) and for future use in modeling the quantitative structure-activity relationships (QSAR) of Army ENMs.

Task 2: Describe a tiered strategy for toxicity testing

Objectives

Mammalian toxicity studies of respirable particles identified by the Army will be conducted using a three-tiered screening system taking into consideration mode-of-action (MOA) of the nanomaterials. The overall goals of the toxicity testing studies are to (1) conduct hazard identification toxicity studies *in vitro* and *in vivo* on a battery of nanomaterials identified in the TEARR system, (2) determine if sets of high throughput *in vitro* assays will be of value in predicting *in vivo* toxicity, and (3) to determine if a battery of predictive *in vitro* assays correlates with a specific MOA category in order to develop reliable alternative toxicity testing strategies. The tiers increase in complexity of living systems and exposure models, and are defined as follows: (1) *in vitro* studies for evaluation of potential pulmonary toxicity of nanomaterials; (2) *in vivo*, bioassay screens to evaluate acute and subacute pulmonary and extrapulmonary toxicity of nanomaterials; and (3) *in vivo* inhalation studies addressing mammalian subacute, subchronic, and chronic toxicity of nanomaterials. Six aims have been established in accordance with the tiers that outline toxicological endpoints relevant to pulmonary exposures to nanomaterials, which in turn are dependent on the physico-chemical characteristics of the material.

Background on MOA Categorization

It is well understood that nanomaterials possess physico-chemical properties that differ from larger particles of the same composition and that these properties influence toxicity of the materials. These properties include, but are not limited to, size, shape, structure, chemical composition, surface reactivity, and solubility, and ultimately contribute to the MOA of nanomaterials in biological systems [Maynard and Kuempel 2005; Kuempel et al., 2012; Nel, 2013; Braakhuis et al., 2014]. Nanomaterials characterization (of a key set of physico-chemical and toxicological properties) allows estimation of the initial MOA category and selection of the recommended testing paradigm to further evaluate the toxic potential of the material. Kuempel et al. [2012] have summarized the particle categorization scheme. Briefly, most nanomaterials may fit largely into one of the following MOA categories which are ultimately a factor of their chemistry: poorly-soluble surface reactive or non-reactive materials, soluble materials, or high-aspect ratio (fibrous) materials. Toxicity of poorly-soluble particles will depend largely on primary particle size/density and the properties of the particles surface that contribute to its reactivity.

Surface reactivity is defined by the degree of radical generation at the particle surface and the ability of this property to affect the biological environment, where higher surface reactive particles are found to induce greater toxicity [Braakhuis et al., 2014; Donaldson et al., 2001]. Cell membranes are negatively charged so the degree of interaction between a particle and a cell surface or the membrane of an organelle may be influenced by the zeta potential of a nanomaterial [Roser et al. 1998]. It has been shown that surface charge influences the toxicity of poorly-soluble particles. For example, in a panel of 15 metal oxide nanomaterials, the acute pulmonary inflammation was correlated with zeta potential in acidic media for low solubility particles [Cho et al. 2012]. Additionally, Bhattacharjee et al. [2010] demonstrated that particles with positive surface charge are more readily taken up by cells and tend to produce more ROS in phagocytic cells. Poorly soluble, low surface reactivity particles are generally considered to be low toxicity, and the MOA is highly dependent on the size and surface area [Duffin et al., 2007] of as given mass of particles deposited in the proximal alveolar region of the lungs [Donaldson et al., 2008]. The alveolar (pulmonary) region has the largest surface area of any region in the lungs, since this is where gas-exchange occurs; it also has the slowest particle clearance rate (via alveolar macrophages to the tracheobronchial mucociliary escalator). Particles that are not cleared may be free to interact with the alveolar epithelial cells, and have a higher probability of penetrating into the lung interstitium where they may be retained or translocated to the lymph or blood circulatory systems. The toxicity of soluble nanomaterials is dependent on the given elemental ion released into solution and the ability of this ion to interact with biological systems; therefore, toxicity is highly dependent on the chemistry of the particle and the dissolution rate of the material [Brunner et al. 2006]. Dissolution rate of the material is, in turn, determined by a number of factors including particle size, surface coatings, stability (chemical bonds), and the physiological solution the material is dissolved in (saline, lung surfactant, phagolysosome solution, gastric secretions, etc.) [Braakhuis et al., 2013]. High-aspect ratio nanomaterials (HARNs) are defined as nanomaterials that have a length-to-width or diameter ratio larger than 3 and at least one dimension in the nano-scale range (< 100 nm) [Donaldson et al., 2011]. They are frequently referred to as fibrous materials due to their shape and include nanorods, nanowires, nanowhiskers, nanotubes, and nanofibers with aspect ratios ranging from 3:1 to > 1000:1. These materials can be similar in chemistry to the materials in the other MOA categories discussed above with the primary defining parameter of toxicity being shape (length and width). In combination

with their length and width, durability/ biopersistence of the material is a major contributing factor to the MOA of toxicity.

Study Design

The goals of the proposal will be addressed in a tier system. The first tier will focus on the portal of entry of the nanomaterial and utilize *in vitro* methods to determine potential for pulmonary toxicity and potency [Oberdorster et al., 2005]. Studies may also be conducted on target organ tissues cultures which may include endothelium, blood, heart, liver, kidney, spleen, and nervous system [outlined in Oberdorster et al., 2005]. For the purpose of this proposal, those studies are not described, but could be performed pending the findings of the extrapulmonary *in vivo* studies. For the proposed studies, particles will be grouped by potential MOA based on physico-chemical characterization, and tested in these groups using a designated battery of *in vitro* methods that examine cytotoxicity, genotoxicity, inflammation, oxidative stress, and fibrogenic responses across a variety of lung cell culture lines, and potentially extrapulmonary cell culture systems. Reference materials that are well-studied and understood to be representative of a given MOA [Kuempel et al., 2012] will be incorporated into the toxicity testing paradigm to determine which tests are the better predictors for toxicity for a particular MOA group at the *in vitro* level. The second tier is a systems approach that consists of bioassay screens to evaluate acute and subacute pulmonary and extrapulmonary toxicity in response to a range of doses of a given nanoparticle at several time points post-exposure. This tier utilizes single or repeated intratracheal instillations (rats) or pharyngeal aspiration (mice) of the materials as routes of exposure. Endpoints in the second tier will address potency related to pulmonary toxicity, including inflammation, lung injury, airway hyper-reactivity, altered pulmonary immune responses, oxidative stress, and disease, such as fibrosis or cancer. It is well-established that pulmonary exposure to particles produces extrapulmonary effects and that these organ systems outside of the lung can be more sensitive to the particle effect than the lung itself. Therefore, in addition to pulmonary responses, systemic toxicity will also be evaluated in the same animals including cardiovascular, neurological, and immune toxicity. The second tier studies will be designed to either incorporate reference materials at a given relevant dose to the study, or parallel existing studies on a reference material for comparative purposes. Pending the findings of tier 2 studies, a third tier of testing may be employed for a given particle. The third tier involves more complex inhalation studies, including

subacute, subchronic, and chronic exposure models. Based on the comprehensive tier two testing, a limited number of particles, as well as a limited number of time points and doses, will be selected for tier three testing to further evaluate the potential for the development of pulmonary and extrapulmonary disease. In addition to the hazard identification data that these studies provide, correlation of the findings of the tier two and three studies for particles in a specific MOA to the results of the *in vitro* battery testing in tier one will allow investigators to establish acellular and in vitro predictive models of toxicity based on particle physico-chemical properties, and ultimately MOA.

Aim 1: Characterize the material in powder and in water-based aqueous solutions for identification of potential MOA category (Tier 1)**.**

Physico-chemical properties of nanomaterials will be characterized in order to establish correlations of toxicity with MOA categories. Below is a list of key properties for categorization and commonly used methods for measuring the given property. Few, if any, standard methods are available for characterization of nanomaterial properties in their as-received (powder) form. At least 28 different lists of physico-chemical properties deemed important for nanomaterial characterization have been developed by various groups [Stefaniak et al., 2013]. The only property that appears on all 28 of these lists is surface area (or specific surface area). The next most frequently cited properties are elemental/molecular composition (bulk), surface chemistry; particle size, size distribution, morphology/shape/form, surface charge; agglomeration/aggregation state; crystal structure; surface reactivity; and solubility (water). Other cited properties of interest included powder density and dry/wet dispersability (hydrophobicity/hydrophilicity). It is important to note that many of these properties are ill-defined or qualitative, and hence, cannot be traced to standard units.

The first set of measurements is designated for the powder form of the material. Many of the techniques are also applicable to the primary particles synthesized through wet chemistry processes as well. It is equally important to understand the physico-chemical properties of the material in the vehicle in which it will be delivered *in vitro* or *in vivo*, most of which are water-based. Frequently, preparation of the particle in the vehicle will involve manipulations, such as sonication, to prevent the nanomaterials from settling out of the suspension before delivery.

Additionally, environments within cells and tissues can range in pH; therefore, evaluating dissolution rate at several different pHs is also of value. These factors should be taken into consideration when performing the aqueous characterization. If applicable, international standard units are noted in square brackets and conventional units in parentheses for each characteristic listed below. Following characterization, particles will be classified by the "expected" MOA based on the physico-chemical properties of the materials.

Characterization of Powder (or Primary) Form:

Primary particle size [m], (nm or μm) - transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), and atomic force microscopy (AFM)

Bulk Chemical Composition [kg/kg or mol/mol], (%) – Inductively coupled plasma (ICP)–mass spectrometry (MS) or ICP-atomic emission spectroscopy (AES) and secondary ion mass spectroscopy; instrumental neutron activation analysis (INAA); x-ray methods (diffraction (XRD); proton induced emission spectroscopy); Raman; or ultraviolet visible spectroscopy (UV-vis) and infrared (IR) [Note: these last two methods are used to characterize surfaces, but may also be useful for bulk chemistry analyses];.

Density [kg/m^3], (g/cm^3) – helium pycnometry (powder)

Shape [none] - TEM, FESEM, AFM, UV-vis

Structure - Crystallinity XRD, electron diffraction

Note: Crystallinity includes the term 'crystallite size' which has units of m though structure is a qualitative property and no unit can be assigned.

Porosity [m^3/m^3 (m^3/kg)], [cm^3/g] – gas adsorption, mercury intrusion

Surface properties: Specific Surface Area [m^2/kg, m^2/m^3], (m^2/g) - isothermal gas adsorption of powder

Note: a protocol for determination of metal oxide nanopowder specific surface area exists [Stefaniak and Hackley 2013]

Surface Reactivity [none] - electron spin resonance (ESR), alterations following UV light exposure

Note: Redox potential has SI units of V and surface energy has SI units of J/m^2 but 'reactivity' is a qualitative property

Surface Charge - titration

Note: Charge and surface charge refer to the charge that arises from the adsorption or desorption of protons on hydroxylated sites on a nanomaterials' surface and is not equivalent to zeta potential. The property 'surface charge density' has SI units of C/m^2

Surface chemistry [mol/m^2], (%) – x-ray photoelectron spectroscopy (XPS), UV-vis, Auger spectroscopy

Hydrophobicity/Hydrophillicty [none] – shake-flask method, dye adsorption tests, contact angle measurement

Endotoxin [none], (CFU) – Limulus Amebocyte Lysate Assay

Characterization of Aqueous Form:

pH of nanomaterial suspension - H^+ ion selective electrode and volt meter; in water and as prepared for delivery *in vitro* or *in vivo* (saline, phosphate buffered saline (PBS) cell culture media, and/or dispersion media)

Note: suspension pH is not a property of the nanomaterial *per se*.

Solubility (dissolution rate) [mol/L, kg/kg, kg/m^3], (%) - in water and delivery vehicle, at neutral and acidic pH of 1 and 4 to 5, and at room temperature and 37 C°– solid separation by centrifugation, filtration, or chemical techniques (more experimental techniques combining static light scattering, UV-vis, and refractometry have been utilized, as well as, fluorous-phase ion-selective electrode sensing systems)

Agglomerate/Aggregate size [none] - in water and vehicle at neutral and acidic pH – dynamic light scattering (DLS), zeta potential [Note: DLS is subject to drying artefacts, in which case it would be uncertain whether the particles were agglomerated in suspension or dried in that configuration].

Note: in nanotechnology, 'agglomeration' has been used to infer reversibility with weak physical forces dominating, while 'aggregation' infers irreversibility with strong and rigid connections between the constituent particles (e.g., fused crystallites)

Zeta potential [V], (mV) – measured as electrophoretic mobility in water and in vehicle

Note: Zeta potential refers to the shear-plane charge near the surface of a nanomaterial in suspension and is not the same as surface charge

Endotoxin [none], (CFU) - in the nanomaterial delivery vehicle suspension – Limulus Amebocyte Lysate Assay

Aim 2: *In vitro* systems analysis of nanomaterials by MOA category to assess potential pulmonary toxicity and correlate mode-of-action with toxicity in vitro (Tier 1).

Following particle characterization, particles will be grouped by potential MOA based on physico-chemical characterization. Toxicity for all MOA groups will be evaluated utilizing the same battery of i*n vitro* techniques and cell lines while incorporating appropriate reference materials in the testing, and will address cytotoxicity, genotoxicity, inflammation, oxidative stress, and fibrogenic responses. Table 2-1 is a composite of several possible tests for each category of toxicity parameters that have been previously utilized in nanoparticle toxicity characterization studies. The test battery will be selected from Table 2-1 upon finalization of the selection of nanomaterials to be evaluated, with the understanding that a given nanomaterial may cause interference with colorimetric assay detection systems, or other assay mechanisms due to the particles physico-chemical properties.

The zebra fish model has been shown to have utility as a high throughput *in vivo* screen for general toxic response such as cytotoxicity, genotoxicity, or developmental toxicity. This model could potentially be correlated to a number of different cell culture lines and mammalian *in vivo* outputs. However, its utility as a model for predicting pulmonary toxicity is not established (and would require a significant validation effort, if a scientific basis for pursuing that approach is demonstrated).

Table 2-1: Toxicity Tests for Consideration for *In Vitro* Toxicity Testing Battery

Toxicity Parameter	Test Category	Assay Options
Cytotoxicity	Membrane Integrity	Vital and Exclusion Dyes, LDH Assay, Protease Assay
	Reduction Enzyme Activity	MTT, WST-1
	ATP Content	Luciferase Assay
	Cell Growth/Proliferation	BrdU, CyQUANT®, MTT, WST-1, CellTiter 96®
Inflammation	Cytokine Production	ELISA (IL-1b, IL-6,IL-8, IL-10, IL-12p70, IL-18, MCP-1, MIP-2, TNF-a)
	Cell Inflammatory Protein Content	Western Blot
	Inflammatory Gene Expression Changes	RNA-Analysis, RT-PCR Array
Oxidant Response/Oxidative Stress	Reactive Oxygen Species	Chemiluminescence, intracellular dyes (DCFH, DHE), OxyBurst Assay®
	Free Radical Production	ESR
	Reactive Nitrogen Species	Greiss Reagent Assay, Peroxynitrite Assay
	Lipid Peroxidation	LPO Assay
	Antioxidant Depletion	Total Antioxidant Assay
	Oxidative Stress Gene Expression Changes	RNA-Analysis, RT-PCR Array
Genotoxicity/Carcinogenicity	Cell Transformation	Colony Forming Assay
	Metastatic Potential	Cell migration/invasion
	Apoptosis/Necrosis	TUNEL Assay paired with alamar blue or BrdU
	Mutagenesis	Micronucleus Assay (MNvit)
	Chromosomal Damage/Abnormalities	In situ hybridization, FISH, COMET assay
	Kinetichore Morphometry	Immunolabeling mitotic spindle and motor proteins
	Cytokinesis	Cytokenisis block (CytoB)
	Cell Cycle Analysis	Cell Cycle Arrest Assay
Fibrogenic Response	Fibroblast Proliferation	MMT, WST-1, CyQUANT®, CellTiter 96®, BrdU
	Collagen Production	Sircol Assay, Sirius Red Staining
	Tissue Remodeling and Collagen Stimulating Proteins	TGF-b, MMPs, TIMPs
	Fibrotic Gene Expression Analysis	RNA-Analysis, RT-PCR Array

Studies will be designed to establish dose-response curves for the nanomaterials that cover a range of responses including the no observed adverse effect level (NOAEL) and low observed adverse effect level (LOAEL) response. Tests will be performed on 3 human cell culture lines (primary rather than immortalized where available) representing the major cell types in the respiratory tract, including small airway epithelial cell (primary SAEC; immortalized BEAS-2B or hTERT), macrophage/monocyte (immortalized THP-1 cells), and fibroblasts (primary NHLF, immortalized CRL1490). Rat or mouse alveolar epithelial cell culture lines may provide a better alternative to the human cancer cell line commonly used for alveolar epithelium models (A549), and may serve as a fourth cell culture model system for toxicity evaluation. Exposure durations and endpoints post-exposure will vary based on the cell line and the assay.

Recommendations for Dosing In Vitro

For materials where exposure data are available:

- Normalize doses in the cell culture models to the relevant (potential) human exposures using deposition models and accounting for the areas or volumes occupied by the cells *in vivo* versus in vitro (lung deposition following an 8 hour exposure to X mg/m^3 is equal to Y mg in the alveolar region of the human lung).
- Normalize the dose in the area of the culture well to the surface area of the alveolar region of the human lung. Set range-finding doses above and below this value (these will be nanogram amounts) to determine NOAEL /LOAEL as well as maximal response while avoiding particle overload responses (responses due to amount of particle rather than the particles properties).
- When exposure data are not available, establish a dose-response curve based on data from previous studies with a reference material from the given particle MOA category and incorporate those values for comparative purposes.

Potential Problems and Considerations for in vitro nanoparticle designs

Dispersions are frequently required for working with and maintaining nanoparticles in a suspension. Dispersion vehicles may vary in effectiveness based on the given material, and interactions of the particles with the dispersion medium may alter toxicity (lipid coating, protein binding). Dispersion mediums commonly utilized and characterized to demonstrate lack of

interference with particle toxicity include a 1.5 % bovine serum albumin or 0.15 mg/ml survanta® (surfactant) for nanoparticle stock preparation which are further diluted in cell culture media [Wang et al., 2010], and a diluted synthetic alveolar fluid for rodent *in vivo* studies (0.6 mg/ml mouse or rat serum albumin with 0.01 mg/ml synthetic dipalmitoyl phosphotidylcholine in phosphate-buffered saline [Porter et al. 2008]. Suspension time and settling rate is an additional variable that can result in different interactions of the nanomaterials at the culture surface, even within a single preparation, and consequently affect toxic responses. Where possible, the settled dose will be calculated based on volume and density relative to time in culture as a variable in comparative toxicity between particles.

Aim 3: Characterize acute and subacute pulmonary and extrapulmonary toxicity over time following a single or repeated pulmonary exposure to various doses of a given nanomaterial *in vivo*. Evaluate lung deposition, clearance, and biodistribution of nanomaterials following pulmonary exposure *in vivo* in relationship to toxicity (Tier 2).

Tier 2 studies will be conducted on selected materials from Tier 1 studies that represent a range of toxicity for a given MOA category. The second tier studies will be designed to either incorporate reference materials at a given relevant dose to the study, or parallel existing studies on a reference material for comparative purposes. The goal of the studies is to establish a dose response curve that encompasses the LOAEL/NOAEL dose, as well as a maximal effect dose for a subacute exposure. Findings from these studies will be correlated with *in vitro* studies to establish the predictive value of the *in vitro* toxicity screening for a given MOA (See Table 2-2). This tier of *in vivo* studies will utilize single or repeated intratracheal instillation (rats) or pharyngeal aspiration (mice) of the materials as the routes of exposure. This method of administration allows for the screening of a broad a range of doses simultaneously with minimal amount of material, and is an effective screening technique for selecting materials and doses for more complex inhalation studies. For these studies, a single-dose exposure is the acute dose and a repeated low dose exposure administered on a schedule (e.g., 1 x per week) for up to one month is considered the subacute dose. Dose ranges will be selected to correlate to *in vitro* studies normalized to lung surface area and /or to parallel existing studies on a given reference material in the particle MOA

category. Parameters of toxicity will be evaluated at multiple time points post-exposure, from 4 hours to 90 days.

For characterization of pulmonary toxicity, the lungs from mice or rats will undergo histopathological and morphometric analysis to assess particle distribution, pleural penetration, clearance and disease, and lung lavage will be performed for biochemical analysis of lung injury, inflammation, and oxidative stress. In addition, molecular changes at the gene expression level correlating to lung injury, inflammation, oxidative stress, and disease will be evaluated in lung tissue. Lung function parameters of airway resistance, compliance, and reactivity will also be measured. Local immune responses in the lung will be evaluated in lung-associated lymph nodes (cell phenotype and gene expression). Extrapulmonary toxicity will also be characterized. Cardiovascular responses will be measured as alterations in (1) hemodynamic parameters (mean arteriole pressure, left ventricular pressure, and heart rate), (2) systemic inflammation and acute phase stress response markers in serum, whole blood, heart, and aorta, and (3) oxidative stress and endothelial dysfunction in the microvasculature will be measured. Kidney and liver will be examined for histopathological alterations indicative of injury or disease. To delineate the systemic immune response, cells from spleens will be harvested and phenotyped, and serum immunoglobulins and immune-related cytokines will be measured. For parameters of neurotoxicity, brain tissue will be collected and histopathology will be performed for various brain regions, and neurochemical (neurotransmitters/biogenic amines) and molecular markers (glial and astroglial) indicative of neuronal inflammation and injury will be measured in brain tissue from specific regions. For nanoparticles where methodology is developed for tracking biodistribution of the nanomaterials, neutron activation or mass spectrometry for metals, and translocation of particles from the lung to the extrapulmonary organs will be assessed and correlated to toxicity.

Table2-2: Suggested Endpoints to Correlate with Pulmonary *In Vitro* Studies:

Pulmonary Toxicity Parameter	*In Vitro* Test Category	Potential In *Vivo* Lung Correlate
Cytotoxicity	Membrane Integrity	Lactate Dehydrogenase (LDH) Activity in Bronchoalveolar Lavage (BAL)
	Reduction Enzyme Activity	
	ATP Content	
	Cell Growth/Proliferation	
Inflammation	Cytokine Production	Polymorphonuclear Cells recovered by BAL; Pro-inflammatory Cytokine Levels in BAL
	Cell Inflammatory Protein Content	
	Inflammatory Gene Expression Changes	
Oxidant Response/Oxidative Stress	Reactive Oxygen Species	Chemiluminescence and DHE/DCFH labeling in BAL cells; Greiss Reagent Assay and Peroxynitrite Assay in BAL fluid; LPO Assay and Total Antioxidant Assay in tissue; RNA-Analysis and RT-PCR Array in Tissue
	Free Radical Production	
	Reactive Nitrogen Species	
	Lipid Peroxidation	
	Antioxidant depletion	
	Oxidative Stress Gene Expression Changes	
Genotoxicity/Carcinogenicity	Cell Transformation	Histopathological Analysis of cancerous/pre-cancerous morphological changes in lung tissue; DNA damage assays (COMET, micronuclei) in Tissue
	Metastatic Potential	
	Apoptosis/Necrosis	
	Mutagenesis	
	Chromosomal Damage/Abnormalities	
	Kinetichore Morphometry	
	Cytokinesis	
	Cell Cycle Analysis	
Fibrogenic Response	Fibroblast Proliferation	Histopathological Analysis of Trichrome Stain in Tissue; Sircol Assay, hydroxyproline assay, and Sirius Red Staining in Tissue; TGF-b, MMPs, TIMPs in BAL fluid; RNA-Analysis and RT-PCR Array in Tissue
	Collagen Production	
	Tissue Remodeling and Collagen Stimulating Proteins	
	Fibrotic Gene Expression Analysis	

To screen for potential adverse effects to various cell types (representing different organ systems that may be exposed to nanomaterials by other routes of exposure such as oral or dermal or by systemic distribution after inhalation) a number of different cell lines could be utilized in predictive toxicology modeling. However, depending on the route of exposure (dermal, inhalation, or gastrointestinal to a lesser degree), the nanomaterials may or may not be in the same form by

the time the material reaches the target organ (if it distributes throughout the body), or may exert its effects elsewhere through signaling cascades rather than direct contact. Therefore, it would have to be taken into consideration that results obtained in those models would come from applying the primary form of the particle to a culture system representing and organ system that may not actually get exposed to that form.

Aim 4: Conduct subacute (< 1 month), subchronic (1-3 months) and chronic (> 3months) inhalation studies for evaluation of the development of pulmonary/extrapulmonary disease and assessment of carcinogenicity of a given nanomaterial based on findings from Tier 2 studies. Establish pulmonary and extrapulmonary biomarkers of toxicity by examining global molecular responses in various organ systems through microarray and gene network analysis based on findings of inhalation studies (Tier 3).

Building on the findings from Aim 3, a high and low potency nanomaterial from each MOA category will be selected for studies conducted under Aim 4. Due to the complexity of inhalation study design, one or two inhalation doses will be selected based on dose-response data collected under Aim 3 and/or based on known field measurements of the nanomaterial in the workers' airspace. Subacute, subchronic, and chronic exposures will be conducted, and multiple time points from 1-90 day post-exposure will be examined. Endpoint parameters of toxicity will follow those outlined for Aim 3 above.

Because inhalation studies provide the most physiologically relevant exposure scenario, biomarker and cancer studies (which are costly and require an extensive amount of time beyond the scope of high throughput *in vivo* toxicity screens) will be conducted under Aim 4. For the inhalation studies described above, whole blood and lung tissue will be collected to evaluate gene expression profiles. Genome array (rat) or microarray (mouse) platforms will be used to identify potential biomarkers of disease. The collected array of datasets will be measured, and networks and functional analyses will be generated through the use of Ingenuity Pathways Analysis (Ingenuity Systems, www.ingenuity.com). The differentially regulated genes, called Network Eligible Molecules, will be overlaid onto a global molecular network developed from information contained in Ingenuity's Knowledge Base. Networks of these molecules will then be

algorithmically generated based on their connectivity, which in turn may help identify biomarkers that appear early in exposure as predictors of potential disease development.

A separate study design for inhalation exposures to determine carcinogenicity will be required. Cancer is a multistep process involving initiation (causing a change in heritable change in DNA), promotion (proliferation of the DNA damaged cells into clones), and progression (neoplastic development of karyotypic instability and neoplasms) [Pitot et al., 1989; 1993; Malkinson et al., 1997]. A complete carcinogen acts at all three stages. To examine the potential for carcinogenicity of a material we will utilize a two stage initiation/promotion protocol in a mouse model to determine whether the compounds act as complete carcinogens and/or promote the growth of cells with existing DNA damage [Sargent et al., 2014]. The two-stage initiation-promotion model for lung cancer described by Rondini et al. [2010[, will be used for the proposed studies. In this model, mice will be exposed to methylcholanthrene (MCA) to damage the DNA (initiation) followed by treatment with a compound which increases the proliferation of the DNA damaged cells (tumor promoter). The B6C3F1 mouse will be used for the studies due to the wealth of data on the longevity, spontaneous tumor incidence, as well as the susceptibility of this strain to carcinogenic compounds. The B6C3F1 mouse is the strain used by the National Toxicology Program (NTP) to evaluate chemicals for potential carcinogenicity. Mice will be treated with an intraperitoneal (i.p.) injection of DNA damaging agent MCA reconstituted in corn oil, corn oil vehicle, or to the suspected initiator. One week following MCA or corn oil treatment, mice will be exposed to the test compound, the positive control vanadium pentoxide, or filtered air by inhalation for 3 months (subchronic exposure). At 14 months after the inhalation exposure, gross lung tumor counts and tumor size will be evaluated in all mice. The lungs, tracheal bronchial lymph nodes, the diaphragm and any tissue masses will be collected at sacrifice and analyzed by histopathological methods to determine the number of carcinomas as well as alterations indicative of injury and disease. Tumors of 4 millimeters or greater will be cut in half, snap frozen, and analyzed for tumor specific protein markers.

Task 3: Suggest methods to evaluate dustiness of nanoparticles

"Dustiness" is defined as the *propensity of a material to generate airborne dust during handling* [Liden 2006]. The formation and emission of dust during the handling of materials (e.g., ENMs) typically depends on the quantity and physical form of the material, the moisture content of the material, the nature of the adhesive forces binding the particles of the material together, and the characteristics of the handling task (the amount of energy imparted to the material). A number of standardized laboratory tests have been tried to replicate mechanisms of dust generation encountered in the workplace [Plinke et al. 1992; Boundy et al. 2006; Brouwer et al. 2006; Evans et al. 2013]. Since dustiness is a relative term and measurement obtained will depend on the test apparatus and various environmental variables it is unlikely that any single method of dustiness testing can represent and mimic all of the various types of processing and handling operations encountered in the workplace. However, controlled laboratory testing of dustiness can provide interim guidance as to the potential relative severity of the airborne release of particles (including nanoparticles) in occupational settings. Testing for dustiness requires the application of sufficient energy to the material to liberate some fraction of loosely bound preexisting primary particles and agglomerates from the bulk material (powder) but not enough to divide the primary particles [Evans et al. 2013]. The more energetic the testing protocol the greater the fraction of airborne dust is liberated from the powder. The object of the test is to mimic the energy supplied at typical processes or job tasks so that an assessment of the potential exposure risk can be made. Such assessments, together with information on the potential hazard (toxicity) of the test material can form the basis for identifying possible exposure control methods (e.g., for use in control banding) [Paik et al. 2008].

A variety of test methods have been used to measure dustiness [Plinke et al. 1991, 1995; Chung and Burdett 1994, Breum 1999; Boundy et al. 2006; Brouwer et al. 2006]. To date, no clear relationship has been established linking inhalation exposure to dustiness measurements as determined by any of these methods. In addition, many of these methods require the use of relatively large quantities of powder (10^2-10^3 grams per test). These quantities of material make it difficult to test nanoscale powders due to their higher costs. The traditional methods for determining the dustiness of powders typically require the use of either the 'f*alling powder'* or 'r*otating drum'* method*.* The continuous '*falling powder'* method involves the dropping of a bolus of particles from a specified height in a chamber; the particles are aerosolized by the

countercurrents generated during the fall or by the countercurrents generated by the impact of the bolus at the bottom of the fall [Plinke et al. 1991, 1995; Heitbrink et al. 1992]. The '*rotating drum'* method rotates the powder in a drum that has internal baffles so that the substrate angle periodically increases causing the powder to fall and aerosolize [Breum 1999; Chung and Burdett 1994]. These two reference methods are currently cited in European standard EN 15051 for dustiness testing [CEN 2006]. In 2006, a qualitatively different method using a Venturi dustiness device was used to test pharmaceutical powders [Boundy et al. 2006]. The Venturi dustiness device permits the use of small quantities of powder (~5 mg) which reduces the potential exposure to potentially toxic pharmaceutical substances. The powder is dispersed into a chamber through a holding tube (containing the powder) at a volumetric flow rate of 60 L/min. Aerosolization of the powder occurs via aerodynamic lift and pneumatic drag mechanisms acting on the powder. Particulate velocities are reported to be one to two orders of magnitude larger than the '*falling powder'* and '*rotating drum'* methods. The method involves more aggressive air flows than those typically encountered in many workplaces but does mimic energetic dust dispersion activities that use compressed air.

NIOSH researchers [Evans et al. 2013] are investigating the dustiness of several types of ENMs using the Venturi dustiness test used by Boundy et al. [2006] to ascertain the viability of this method for estimating the size fraction (total and respirable) of dispersed particles and agglomerates. The assessment is intended to provide data that can be used for: 1) ranking fine and nanoscale powders according to their airborne dust generating abilities, 2) developing an index for estimating their potential flammability/combustibility, and 3) estimating potential ENM exposures for control banding (e.g., CB Nanotool). To date, 27 different types of fine and nanoscale powders have been evaluated for dustiness [Evans et al. 2013]. These 27 materials included single- and multi-walled carbon nanotubes (MWCNTs), carbon nanofibers (CNFs), carbon blacks; oxides of titanium, aluminum, silicon, and cerium; metallic nanoparticles (nickel, cobalt, manganese, and silver), silicon carbide; nanoclays; a mixed metal oxide, lithium titanate, and Arizona road dust (an internal standard to verify the operation of the equipment). For eight of the materials, total and respirable dustiness was studied as a function of relative humidity at 20, 50, and 80%. For most of the tested materials relative humidity had little detectable effect on dustiness. Also, no one material class of ENMs was found to be excessively dusty (i.e., significantly more dusty than the others), conversely, no one material class was found to be relatively 'dust

free'. As anticipated, respirable dustiness was always less (about 2/3 less) than total dustiness for all tested materials. Furthermore, total and respirable dustiness appear to be correlated in a linear relationship although this result may have been due to the selection of ENMs. It was also noteworthy that no aerodynamic particle size mode below 100 nm was observed for any of the materials tested, despite the relatively energetic dispersal mechanism employed with the Venturi testing device. These findings suggest that it is unlikely that a substantial sub-100 nm fraction of these ENMs would be encountered in the workplace and that engineering controls frequently used for preventing worker exposures to fine powders would be applicable when handling nanoscale powders.

Task 4: Suggest methods to evaluate worker exposure (particle size distribution, count, mass, etc.) during processing and incorporation into materials and soldier exposure in the field

Despite the progress made in recent years with measurement techniques and strategies for the workplace exposure assessment of ENMs, there remains some uncertainty as to an appropriate exposure metric to use when measuring exposures to ENMs. In addition, the assessment of airborne ENMs remains challenging due to the lack of portable and personal instruments that are selectively sensitive to ENMs against a background of intermittent naturally occurring or incidental nanoparticles (e.g., from propane or diesel powered forklifts, combustion activities such as gas-fired heaters, welding/soldering fumes). Thus, measurement techniques and measurement strategies have to be optimally combined to allow sensitive and cost effective determinations of airborne ENMs. Traditionally, the measurement of mass concentration has been regarded as the most appropriate exposure metric associated with health effects of particle exposures. However the appropriateness of using the mass concentration metric for ultrafine particles remains questionable, as particle number and surface area concentrations have been proposed as more suitable alternatives for ENMs. For most nanomaterials in use, occupational exposure limits (OELs) have not been established for the nanoscale form of the material. The absence of OELs and the uncertainty of the health risks have led to the use of control banding approaches for assessing the risk and implementing exposure control measures for ENMs [Ramachandran et al. 2011]. While control banding is useful in the interim, a systematic and ongoing monitoring of workers airborne exposures are needed to ensure that exposure control measures are effectively reducing worker exposures.

There has been a number of exposure measurement strategies proposed that can be used to qualitatively and quantitatively evaluate workplace exposures to ENMs [Brouwer et al. 2009; Methner et al. 2010; Ramachandran et al. 2011]. An important first step in applying any exposure measurement strategy is the need to first develop an inventory of materials used and have knowledge about the processes and job activities where these materials may place workers at risk of exposure. This initial assessment of the workplace should attempt to answer the following questions:

- Is the material dusty and is the process or job activity likely to generate dusts or aerosols of ENMs?
- What is the quantity and physical state of the ENM at each stage of the work process (i.e., dry powder, suspension or liquid, embedded or bound in other materials)?
- Does the process include the handling of the dry powder form of the ENM, the mixing or sonication of a suspension containing ENM, or the cutting, grinding or other activity where high energy is imparted to an ENM-containing material?
- What are the potential routes of human exposure (e.g., inhalation, dermal, ingestion)?
- What is the likelihood of exposure occurring during normal routine work, maintenance, waste management, and accidental releases?
- How often is exposure likely to occur, for example, continuously over a working shift, intermittently, or rarely?
- Are engineering controls and/or other measures to reduce exposures being used?

This initial assessment of the workplace can be used to determine the number of workers potentially exposed and a qualitative assessment as to the workers and processes with the highest potential for exposure.

Tier 1 exposure measurement strategy

The Army may want to consider initiating a multi-tiered approach to the measurement and control of workplace exposures to ENMs. Given the current uncertainties of the health risks and absence of information on worker exposure to ENMs the initial exposure assessment effort should focus on identifying processes and job activities that are potential sources of exposure **[Tier 1 exposure assessment]**. NIOSH has used such a strategy for identifying and measuring ENM emissions at processes and job activities (e.g., laboratories, pilot operations) where typically small quantities of ENMs or ENM-enabled materials are handled [Methner et al. 2010]. The strategy uses a multifaceted approach for identifying and characterizing exposures using different sampling techniques. These sampling techniques include a combination of direct-reading handheld instruments (i.e., condensation particle counter (CPC) and optical particle counter (OPC)) to measure particle concentrations as well as the use of filter-based air samples for particle identification and characterization. Because of the uncertainty of the size range of particles being released into the air, the simultaneous use of the CPC (measures particle sizes 10-1000 nm) and

OPC (measures particle sizes 300-10,000 nm) can provide a semi-quantitative indication of the nature and magnitude of emissions at each process and job activity. The use of filter-based air sampling provides an opportunity to collect additional information about the exposure including the chemical composition and mass concentration, as well as information on particle morphology (shape, size, degree of agglomeration) when analyzed by transmission electron microscopy (TEM) or scanning electron microscopy (SEM). Filter-based air samples can be collected in areas of the work environment where exposure to ENMs are suspected and/or as a personal breathing zone (PBZ) sample collected on a worker who might be potentially exposed to the ENM of interest. This exposure measurement strategy can be useful in identifying sources of potential exposure and for determining the need for exposure controls.

Background workplace particle concentrations are an important consideration when attempting to identify a source of ENM emission since it can potentially interfere with the interpretation of ENM particle number concentrations. The accounting for background and incidental particle concentrations can be accomplished in several ways that are often situation specific. One option is to measure airborne particle number concentrations using the CPC and OPC by beginning the data logging before the processing or handling of the ENM begins. If this initial background particle number concentration is high, an effort should be made to identify the source of the particle exposure (this might include measuring outside ambient particle concentrations or the intake air into the work place). Because background particle number concentrations can be variable, it is necessary to continue to measure particle number concentrations continually using the data logging mode before and after each process/job activity and comparing that data to measurements made during that particular process or job activity. Detailed notes on the timing of the activities occurring is necessary for the data interpretation. Within a closed environment such as a clean room, background samples (i.e., direct reading instruments) should be collected inside the clean room, but as far away from the emission source as possible. Measurement data collected in this manner can then be used to identify processes, job activities, locations, and personnel for subsequent filter-based air sampling to better characterize the exposure.

Tier 2 exposure measurement strategy

NIOSH has continued to refine its exposure measurement strategy for ENMs into a more comprehensive method aimed at assessing worker and workplace exposures. This strategy builds

upon the knowledge gained from the exposure information gathered in the T**ier 1 exposure measurement strategy**. Use of this **second tier** exposure measurement strategy can provide the Army with more comprehensive exposure data that can be used for implementing and improving risk management efforts. This **Tier 2** effort is aimed at better characterizing worker exposures by placing more emphasis on time-integrated filter-based samples (i.e., elemental analysis and electron microscopy) collected in the worker's breathing zone (full shift and task specific) as well as the collection of area samples to develop job exposure matrices. Real-time instruments (e.g., CPC, OPC) operating in a data logging mode are still used to evaluate peak exposures of workers and for characterizing ENM emissions (e.g., particle size and concentration) at processes and job activities.

Right now, recommended exposure limits (RELs) exist for only three nanomaterials [NIOSH 2011, 2013]:

- ultrafine titanium dioxide (TiO_2) REL = 300 μg/m^3;
- carbon nanotubes (CNT), and carbon nanofibers (CNF) REL = 1.0 μg/m^3 as elemental carbon (background-corrected)

The RELs for these materials are expressed as mass per volume, on a respirable fraction. Thus, these nanomaterials should be sampled on a mass per volume basis for comparison.

Commensurate with the measurement of airborne exposures, efforts should be made to ascertain the potential migration of ENMs outside of the work area on both equipment and other surfaces. The assessment of potential contamination is important especially when the use of ENMs increases and as more persons become involved in handling ENMs and ENM-enabled materials. Wipe samples can be collected using "NIOSH Method 9102, Elements on Wipes", on surfaces that workers frequently come into contact with (e.g., doorknobs, computer keyboards) and can provide indication of potential dermal ENM exposure and transfer while positive wipe samples for ENMs collected on horizontal surfaces could indicate the airborne migration of the ENM due to ventilation or engineering control problems. "Concentration Mapping" is another technique that can be used to identify spatial and temporal variability of the ENM concentration distribution in the workplace [Ramachandran et al. 2011]. This technique can be used to identify contaminant sources and as a quantitative tool to prioritize the implementation of exposure controls and for determining sampling locations. As knowledge is gained on sources of emissions and the

likelihood of worker exposure, the collection of information on worker job responsibilities and work practices (e.g., routine versus non-routine, frequencies and duration of potential exposures) should be considered for the possible future development and implementation of medical and hazard surveillance programs.

Exposure data gathered in Tier 1 and/or 2, along with available information on the hazard potential of the ENM, can be used to place the ENM in an appropriate exposure control band as described in Task 5.

[Note: NIOSH is in the process of publishing its updated strategy for measuring exposures to ENMs and will provide the Army with a copy of the manuscript when it's published]

Tier 3 exposure measurement strategy

As knowledge is gained about the toxicology of ENMs (i.e., biological MOA, relevant physico-chemical properties), risk assessment efforts can be undertaken to better describe exposure response relationships. This assessment may eventually lead to the derivation of safe levels of exposure or occupational exposure limits (OELs) that can be used by health professionals, employers, etc. in recommending risk management guidance. Knowledge about the seriousness of the health concern associated with exposure to an ENM should trigger more diligence in the control of exposures so that worker exposures are maintained at the lowest possible concentration or below a designated OEL. To attain such assurances a more comprehensive and systematic assessment of worker exposures is required. The selection of an appropriate exposure measurement strategy will depend on a number of factors, including the number of workers potentially exposed to the ENM and information on the variability in airborne concentrations (i.e. day-to-day and worker-to-worker exposure variability). Several types of exposure measurement strategies are available that can help provide statistical confirmation that workplace airborne exposures are being controlled at a target concentration [NIOSH 1977; Corn and Esmen 1979; Leidel and Busch 1994; Rappaport et al. 1995; Lyles et al. 1997; Bullock and Ignacio 2006; Ramachandran et al. 2011; McNally et al. 2014]. These strategies can be tailored to the specific workplace depending on the number of workers, complexity of the work environment (e.g., process type and rate of operation, exposure control methods, physical state and properties of the

material) and available resources. One approach for determining worker exposure would be to initially target groups of similarly exposed workers [Corn and Esmen 1979; Leidel and Busch 1994; Clerc and Vincent 2014]. This initial sampling effort may be more time efficient and require fewer resources for identifying workers with exposures that exceed a pre-specified airborne concentration (e.g., OEL). However, this measurement strategy may produce incomplete and upwardly biased exposure estimates if the exposures are highly variable [Symanski et al. 2006; Kromhout 2009]. Therefore, repeated measurement on randomly selected workers may be required to account for between- and within-worker variation in exposure concentrations [Rappaport et al. 1995; Lyles et al. 1997]. For workplaces and job activities where some exposure measurement data exist as well as some understanding of the exposure variability in the workplace, it may be possible to use a "Bayesian" model that combines expert knowledge with existing exposure measurement data to estimate workers' exposures [Sottas et al. 2009; McNally et al. 2014]. Because there may not be a specific exposure measurement strategy that can be applied to all workplaces and job activities, multi-day random sampling of workers (all workers, if the exposed workforce is small) may be required to have an accurate assessment of worker airborne exposure concentrations.

Task 5: Describe the current hazard and control banding options for nanomaterials

Control Banding Methods

Existing knowledge about the uncertainty of the health risks to ENMs provides a basis for implementing risk management practices that focus on minimizing worker exposures. Control banding is a qualitative risk assessment and risk management tool that focuses resources on determining and implementing exposure controls that are appropriate for the hazard encountered. This approach can be applied in situations where occupational exposure limits (OEL) have not been established (i.e. ENMs) or can be used to supplement OELs, when available. The premise of control banding is that information on the inherent toxicity (i.e., hazard band) of a material can be combined with information on the type and physical properties of the material to categorize the material into established control bands. This information can then be used to suggest the control strategy required to decrease potential exposure and ensure that the material is being used safely.

There are several control banding tools available that can be used to assist in determining the types of exposure control measures that can be applied. The Control of Substances Hazardous to Health (COSHH) Essentials method was developed by the United Kingdom's Health and Safety Executive in 1999 to assist small businesses in protecting their workforce when potentially hazardous materials are in use that may not have OELs [Maidment, 1998]. This method was not specifically developed to apply to nanomaterials, but has provided the methodology by which other control banding tools have been constructed. By inputting characteristics of the material (health hazards based on the chemicals risk phrases, dustiness or volatility of the chemical, and the quantity used or generated) the use of control banding can provide assistance in controlling exposures. Maidment [1998] also stressed the importance of limiting the number of factors in the control banding model so that the model remains simple and able to be used by the target audience (e.g., small business). The COSHH Essentials method places the material into one of four control strategies: 1) does not require any special engineering controls or containment, 2) requires the use of local exhaust ventilation, but does not require an enclosed system, 3) requires the use of industrial closed containment, and 4) refers the business owner to seek specialist advice such as that of an Industrial Hygienist. Based on the unique properties of ENMs and the expanding

new use of them in industry, it has been suggested that control banding strategies may assist businesses in controlling the potential hazards associated with these new materials [Maynard, 2007]. The Control Banding Nanotool was developed by Paik et al. [2008] to determine suggested 'control levels' for nanomaterials. This tool uses both a hazard severity score and a probability score to determine the risk level for the material. The hazard severity determination is based on what is known or unknown about the nanomaterial and the parent material and takes into account information such as the surface chemistry, toxicity, dermal hazard potential, and particle diameter. The probability score takes into account information such as the quantity, dustiness, frequency, and duration of operation. All associated factors are rated based on a point system and the values for both probability and severity are used to determine the risk level and the control measures necessary to control that particular material. The final matrix used in the Control Banding Nanotool to determine the appropriate control band for the material is shown in Figure 5-1.

Probability

Severity	Extremely Unlikely (0-25)	Less Likely (26-50)	Likely (51-75)	Probable (76-100)
Very High (76-100)	RL 3	RL 3	RL 4	RL 4
High (51-75)	RL 2	RL 2	RL 3	RL 4
Medium (26-50)	RL 1	RL 1	RL 2	RL 3
Low (0-25)	RL 1	RL 1	RL 1	RL 2

Control bands:

RL 1: General Ventilation

RL 2: Fume hoods or local exhaust ventilation

RL 3: Containment

RL 4: Seek specialist advice

Figure 5-1. Risk level matrix as a function of severity and probability used in the Control Banding Nanotool. Control bands are based on overall Risk Level. Source: Paik et al. Ann Occup Hyg. 2008. 52:419-428

The GoodNanoGuide (goodnanoguide.org) provides a simple complementary control banding method that utilizes available information on exposure duration, potential for material

aerosolization, and the hazard potential of the material, to assist in selecting appropriate exposure control measures (Figure 5-2).

Exposure Duration	Bound Materials	Potential Release	Free / Unbound
Hazard Group A (Known to be inert)			
Short	1	1	2
Medium	1	1	2
Long	1	2	2
Hazard Group B (Understand reactivity/function)			
Short	1	2	2
Medium	1	2	3
Long	1	3	3
Hazard Group C (Unknown Properties)			
Short	2	2	3
Medium	2	3	4
Long	2	4	4

Exposure Duration Key: Short: < 4 hours/day, 2 days/week Medium: 4 to 6 hours/day, 3 to 5 days/week Long: 6 to > 8 hours/day, 3 to 5 days/week

Release Key: Bound: Nanoparticles in Solid Matrix Potential: Nanoparticles in friable or sol gel matrix Free/Unbound: Nanoparticles unbound, not aggregated

Control Band (Risk Level) Key: 1: General ventiliation and personal protective equipment ("PPE") 2: Engineering controls and/or respirators, additional PPE 3: Containment (e.g., glove box) 4: Seek specialist advice

Figure 5-2. GoodNanoGuide risk level matrix as a function of exposure duration, material release potential, and assigned hazard group. Source: GoodNanoGuide (https://nanohub.org/groups/gng/control_banding)

Control Banding Results

Each of the selected nanomaterials given in Table 5-1 was evaluated using the GoodNanoGuide and the CB Nanotool [Paik et al. 2008; Zalk et al. 2009]. The Stoffenmanager Nano [Marquart et al 2008; Duuren-Stuurman et al. 2012] method was not used since it is a risk prioritization tool that

provides information currently provided in the Money et al. [2013] report and the TEARR database.

Table 5-1. Initial list of selected engineered nanoparticles (ENM) by material class possible benchmark materials within material category

Category[a]	No.[a]	ENM[a]	Initial Biological and Physical-chemical based Mode of Action Category[b]
Carbon-based	5, 10	Carbon nanotubes (CNT)	Fibrous (high aspect ratio) particles
Metals	14	Silver nanoparticles	Higher solubility particles
	23	Nickel nanoparticles	
Metal oxides	34	Titanium dioxide (TiO_2)	Poorly-soluble, low toxicity particles
	33	Silica (SiO_2)	Poorly-soluble, high toxicity particles
	35	Zinc Oxide (ZnO)	Higher solubility particles
	28	Alumina (Al_2O_3)	
Inorganics	37	Tungsten nanoparticles (W)	Poorly-soluble, low toxicity particles[c]
Quantum dots	41	Cadmium selenide (CdSe)	Poorly-soluble, high toxicity particles[c]

[a] From "Table 1. List of ENMs Included in TEARR", p. 6 of RTI report (Money et al. 2013).
[b] Described in Kuempel et al. (2012) and Task 7 of this report.
[c] Tentative category, pending further evaluation.

Relevant occupational exposure scenarios were created to assist in determining potential acute or chronic health risks and the associated controls recommended by each method for each ENM. Inhalation was assumed to be the primary route of exposure, but potential dermal hazard was noted for materials with data indicating a dermal hazard. The exposure scenarios were assumed as either short-term/acute (e.g., 15-30 min, as in short-term exposure limits, STELs) or repeated/chronic (8-hr time-weighted average, TWA) exposures.

In recommending appropriate exposure control measures, it was assumed, at this time, that Army personnel and other workers are potentially exposed to unknown quantities of ENMs being used in small-scale operations (i.e. research and development). Workers exposed while handling a powder form of the ENM for more than 4 hours per day and 5 days per week were considered to have a chronic exposure. The ENM was assumed to be spherical in shape (if not otherwise specified) with a diameter of 40 nanometers. Surface reactivity was assumed to be high due to the

unknown potential for functionalization and because of the high surface reactivity that has been observed for other ENMs. Solubility was determined based on information provided in the TEARR database. If more than one type of solubility was listed, then the ENM was considered to be insoluble. Information was also gathered on the larger counterpart material such as the carcinogenicity, dermal hazard and asthmagen potential. If the larger 'bulk' material had been identified as carcinogenic, a dermal hazard, or an asthmagen, then the ENM was also assumed to create similar health effects. Otherwise, all ENM health data were indicated as unknown. The questions, answers, associated scoring, and supporting evidence for chronic exposure to each material is documented in Appendix A.

The probability score determined by the CB Nanotool for chronic exposure (score 78.75) was the same ("likely") for each ENM (Table 5-2). By decreasing the amount or dustiness of the ENM, the exposure duration will decrease the score and thereby change the exposure control recommendation to one that is less conservative. Likewise, decreasing the exposure time and increasing the amount of the ENM (frequency of operation < monthly, duration of operation < 30 minutes, and estimated amount of ENM used > 100 milligrams), the exposure to the ENM would be considered acute and the probability score would indicate "likely" with a score of 55. By altering the exposure, this would decrease the risk level from that defined by chronic exposure and decrease the need for recommending a more stringent exposure control measure. The severity score varied dependent on the material characteristics and information on the larger "bulk" material. It should be noted that chronic versus acute exposure does not affect the severity score. ENMs that have a specified shape (i.e. CNTs or graphene), or those reported to cause cancer or dermal health effects, increased the severity score. Unfortunately, at this time, limited information exists on the potential health effects of these ENMs. Questions with an answer of "unknown" were assigned the associated "unknown" scoring of 75% of the maximum which prevents the severity score to fall below the "medium" severity range. All reviewed nanomaterials and the associated CB Nanotool scores and recommendations are summarized in Table 5-2.

Table 5-2. Summary of data and recommendations using CB Nanotool.

Category	ENM	MOA Category	Severity Score	Probability Score	Recommendation
Carbon-based	CNT	Fibrous	56	78.75	RL4 - Seek specialist advice
	Graphene		56	78.75	RL4 - Seek specialist advice
Metals	Silver NP	Higher Solubility	46	78.75	RL3 - Containment
	Nickel NP		59.5	78.75	RL4 - Seek specialist advice
Metal Oxides	TiO_2	Poorly-soluble low toxicity	50	78.75	RL4 - Seek specialist advice
	SiO_2	Poorly-soluble high toxicity	51.5	78.75	RL4 - Seek specialist advice
	ZnO	Higher Solubility	46	78.75	RL3 - Containment
	Al_2O_3		47.5	78.75	RL3 - Containment
Inorganics	Tungsten NP	N/A	46	78.75	RL3 - Containment
Quantum Dots	CdSe	N/A	61.5	78.75	RL4 - Seek specialist advice

Using the GoodNanoGuide method (as described in Figure 5-2) and assuming chronic exposure, each ENM evaluated would fall into the "medium" duration exposure category (4-6 hours/day, 3-5 days/week) resulting in an increased potential for the release of unbound (or not aggregated) ENMs during handling and manipulation. By assuming an acute exposure scenario, the exposure duration would change to "short: < 4 hours/day, 2 days/week" with the same increased potential for the release of unbound ENM. The one variable not able to be determined is placement of the ENM in the appropriate 'hazard group'. If for example, all of the ENMs fell into "Hazard Group C" based on unknown properties, then using the table provided, these characteristics would place the ENM (for chronic exposure) in control band (risk level) 4 which recommends seeking the advice of a specialist and the ENM (for acute exposure) would be placed in control band (risk level) 3 which recommends the use of exposure containment. If the reactivity and function information is known

for the ENM ("Hazard Group B"), then the recommended control band (risk level) for both chronic and acute exposure would decrease by one level (risk level 3 – exposure containment, and risk level 2 – engineering controls and /or respirators and additional personal protective equipment, respectively).

Until more information becomes available on the physico-chemical characteristics and health risks to these ENMs, the use of control banding can be used to support and complement other current exposure and risk management practices. Additional information on control banding is available at www.cdc.gov/niosh/topics/ctrlbanding/.

Task 6: Describe controls to be employed to reduce exposure for a given hazard band

One of the best ways to prevent potential adverse effects from exposure to ENMs is to minimize or eliminate exposures early in the design of processes where ENMs and ENM-enabled materials are produced and used. The use of engineering controls can be an effective control strategy for minimizing exposure to ENMs. Well-designed engineering controls can be highly effective in protecting workers when they are properly designed, tested, and routinely maintained and evaluated to ensure maximum efficiency [ACGIH 2010]. There are a number of different types of exposure control systems that can be used depending on the configuration of the process and the degree of exposure control required [ACGIH 2013; NIOSH 2013b]. These systems range from enclosing the process and using automatic handling techniques (i.e., isolating the generation source from the worker); to partial containment using local exhaust ventilation (LEV) equipped with high efficiency particulate air (HEPA) filters. The selection of the appropriate exposure control system should take into account the extent to which the airborne concentration of the material is to be reduced (i.e., below an existing OEL; for use with a given 'hazard band'), the quantity and physical form of the material (e.g., dispersible powder, liquid slurry, contained in a matrix), the task duration, the frequency in which workers come into contact with the material, and the characteristics of the task itself (high energy imparted to the material, e.g., sonication, powered sanding/cutting).

As described in Task 5, "Control Banding Methods", potentially relevant occupational exposure scenarios were used (Figures 5-1 and 2) to assist in evaluating acute or chronic exposures and identifying the risk level and associated exposure controls recommended by each method for each ENM. Inhalation was assumed to be the primary route of exposure, but the potential dermal hazard was also considered when data were available. An initial list of ENMs were selected (see Table 5-1) that could be used by the Army as a benchmark for categorizing the hazard potential of other ENMs (i.e., based on similar biological activity and physico-chemical characteristics) so that appropriate exposure mitigation actions can be initiated. When determining the hazard potential and recommended control band (see Table 5-2), the severity and probability scores for the ENMs were influenced by the lack or limited toxicological data and the absence of exposure information.

Based on the hazard scoring of a select group of ENMs presented in Table 5-2, recommendations for minimizing worker exposure resulted in "seek specialist advice" and exposure "containment". Specialist advice involves informed application of the hierarchy of controls beginning with elimination, substitution, product modifications, process modifications, and equipment modifications. When these actions are not feasible engineering controls such as glove boxes, down-flow booths, and LEV are recommended to minimize process emissions. Exposure containment is considered the highest level of exposure control in the 'containment' hierarchy [NIOSH 2013b].

Due to the lack of information on how ENMs and ENM-enabled materials are being used by the Army, the following discussion focuses on specific exposure control strategies that can be used when handling small quantities of ENMs. Other types of occupational exposure scenarios (e.g., use of large quantities of ENMs, outdoor use of ENM-enabled materials) may require a different exposure mitigation strategy, including the use of personal protective equipment (e.g., respirators) when the use of engineering controls is not feasible.

A number of workplace activities (i.e., processes, job activities) have been reported in the literature that involve the handling of small quantities of ENMs [NIOSH 2013b]. These processes and job activities may be similar to those experienced by Army personnel. Below is a list of activities in which emissions to ENMs have been reported and engineering controls have been used to minimize worker exposure:

- Manual harvesting of the ENM from reactors
- Discharging product into containers
- Manual transfer between processes
- Weighing out of powder
- Emptying of bags and containers
- Mixing or compounding
- Sonication of ENMs
- Cleaning equipment to remove debris
- Changing filters on dust collection systems
- Further processing of products containing ENMs (e.g., cutting, grinding, drilling)

Exposure Source and Control

Many options are available to control worker exposure to ENMs during small-scale material handling operations. The best option for a given process or task depends on several factors including the scale of the handling operation, the physical properties of the ENMs being handled (size, density, wet or dry formulation), work environment (laboratory versus plant, cross drafts, nearby activity), potential 'dustiness' of the ENM, equipment requirements (size of equipment/operation being enclosed), and level of protection required. Independent of the exposure control selected, only the smallest quantities of the ENM should be used when possible and workers should be trained on the safe handling of the ENM and on the proper use of the exposure containment system. Other procedures, such as wiping down and sealing containers before they are removed from the containment, are recommended. The proper positioning of workstations and exposure containment systems away from doors, windows, air supply registers, and aisle ways will help to reduce the impact of cross drafts.

1) ENM reactors (harvesting, cleaning, maintenance)

Emission sources related to reactor operations, harvesting, cleaning, and maintenance are frequently characterized as fugitive or task-based. The approaches that have been used for controlling fugitive emissions from the reactor have primarily been ventilated enclosures. Laboratory fume hoods (i.e., variable air volume hood) and glove box isolators can be used when the reactor is small (typically used in research laboratories). Where the reactors are larger, custom-fabricated enclosures often constructed from a polycarbonate, transparent thermoplastic material, or vinyl curtains have been used to reduce emissions. When designing these types of enclosures, it is necessary to consider reactor access needs, determination of exhaust airflows capable of maintaining a negative pressure (during the opening of access doors), and accommodation of heat loads generated by the process. If the process is heated, the use of canopy hoods may be an alternative as long as the design meets the operational and facility exposure control requirements [ACGIH 2013]. When controlling exposures during operations such as product harvesting and reactor cleanout/maintenance, the use of spot LEV systems (e.g., fume extractor) for exposure containment may be acceptable. Manual harvesting of product materials

may be better suited for higher-level enclosure controls such as a glove box isolator or a specially designed enclosure to provide good capture while minimizing loss of product material.

2) Small-scale weighing, mixing, sonication, and handling small quantities of ENM powders

Small-scale weighing and handling of ENMs (e.g., powders) are common tasks; examples include working with a quality assurance/control sample and the processing of small quantities (mixing, transferring) for downstream use. During these operations, workers may weigh out a specific amount of ENMs to be added to a process such as mixing or compounding. The tasks of weighing the ENM can lead to worker exposure primarily through the scooping, pouring, and dumping of these materials, and should be performed in enclosed containment systems, and when warranted, HEPA-filtered ventilated systems. HEPA filtration has been shown to be effective in capturing nanoscale particles and should be considered in situations where emissions may be likely, where processes are repeated, and where higher quantities are used in a way that may lead to emissions. Many different types of exposure containment systems are commercially available that can be employed to reduce exposure during the handling of small quantities of ENMs; these systems include low flow laminar chemical fume hoods, glove box isolators, biological safety cabinets or cytotoxic safety cabinets. Ductless re-circulation HEPA-filtered containment systems that re-circulate air back into the room from the containment system through a HEPA-filter, can be used for small quantities of ENMs in the absence of any hazardous vapors or gases. However, the use of a ductless re-circulation containment system to control exposure to ENMs must be subject to rigorous evaluation and should only be considered where external venting to a safe place outside is not reasonably practicable.

Some studies have shown that bench top activities such as probe sonication of ENMs in solution can also result in emission of airborne particles [Johnson et al. 2010; Lee et al. 2010]. Producing dispersions by sonication can be a primary operational step, and the assessment of the task should address the sound level exposure as well as the potential exposure to aerosols of ENMs from the sonication. Maintaining the sonication/dispersion process within an enclosure such as a hood can be an effective means for mitigating the noise and aerosol exposure.

3) Changing filters on dust collection systems

When exposure containment systems equipped with HEPA filters are used to contain ENMs and other dusts, these filters will periodically need to be changed. When filters require change-out, the use of integral containment equipment and isolation procedures (such as bag-in bag-out) can reduce maintenance worker exposure. Other general maintenance procedures, such as modifying ductwork or performing fan maintenance will also require appropriate precautions to avoid exposing workers to ENMs settled in the equipment. In cases where full containment of exposure is not possible, the use of respiratory protection may be advisable.

4) Machining (e.g., cutting, grinding, drilling) of materials containing ENMs

Studies have shown that the machining of some nanocomposite materials can result in the release of nanoscale particles in the work environment. An exposure containment system should be used when the nanocomposite material is small; for large nanocomposite materials, engineering controls (e.g., LEV) are available for capturing emissions for most common machining processes [ACGIH 2013]. In some cases, the use of wet suppression techniques during machining has been shown to significantly reduce exposures to nanoscale particles [Bello et al. 2010].

Evaluation of Exposure Controls

Routine evaluation of exposure control systems should be conducted to ensure that they are functioning properly and preventing the release of engineered nanoparticles. This assessment should include the collection of both quantitative and qualitative exposure data to describe the emission. The use of direct reading particle counting instruments (e.g., condensation particle counter, optical particle counter) can provide information (i.e., particle number concentration) about the spatial and time variation in the release of nanoparticles at the exposure source. This exposure information and knowledge about the "background" particle concentration can be used to evaluate the effectiveness of the exposure control. When required, airborne samples should be collected and analyzed by electron microscopy for particle identification (as described in Task 4); this evaluation can also provide information on the physical characteristics of the ENM particle of interest and the presence of other nanoscale particles.

Task 7: Describe methods to evaluate exposure-health effect relationships for selected nanomaterials

Background

Nanotoxicology studies are generating large amounts of data which have potential utility for evidence-based risk management decision-making. Many of these studies provide alternative test strategy (ATS) data, including *in vitro* (cellular) assays and limited *in vivo* studies in a tiered testing scheme Oberdörster et al. [2005] (as discussed in Task 2). Researchers at NIOSH and elsewhere are examining how ATS data can be used to fill data gaps for hazard and risk categorization of ENMs. The approaches being evaluated include linking ATS comparative toxicity data to quantitative risk estimates of benchmark (reference) materials to derive occupational exposure limits or bands (OELs or OEBs) for nanomaterials within physico-chemical and biological mode-of-action categories. Benchmark particles are well-characterized materials for which quantitative risk estimates and health-based OELs are available and which will serve as a point of reference for comparative potency analyses and development and evaluation of OELs or OEBs [Oberdörster et al. 2005; Kuempel et al. 2007, 2012; Nel et al. 2013].

Occupational exposure limits have long provided the health basis for risk management decisions, including the evaluation and selection of engineering controls. Currently, no regulatory OELs have been promulgated for ENMs. Non-regulatory OELs for certain ENMs, developed by non-regulatory governmental agencies or by nongovernmental organizations or individuals, are typically lower (as airborne mass concentrations) than the closest applicable regulatory OELs (Table 7-1). In the absence of OELs, control banding schemes may be used to make exposure control decisions [NIOSH 2009]. These schemes use qualitative hazard and exposure bands (ranging low to high) to derive the control band and associated exposure control options. Further information on control banding schemes for ENMs and their potential application for ENMs is provided in Task 5.

Some hazard banding schemes include quantitative OEBs, which are typically based on limited hazard data from animal studies. OEBs have been used as initial or provisional OELs. In recent years, several ENM-specific control banding strategies have been proposed [Zalk et al. 2009; Duuren-Stuurman et al. 2012; ISO 2012], but the effectiveness of these strategies to protect

workers' health has not been evaluated. Thus, a critical need in occupational safety and health (OSH) is the development of evidence-based criteria for use in deriving either OELs or OEBs for ENMs. NIOSH is working on developing hazard and risk based categories of ENMs based on physico-chemical properties and tiered testing data. The availability of benchmark substances to which the bioactivity of ENMs can be compared is a key element of this framework (Figure 7-1; Table 7-2). The framework for using data from benchmark materials in addition to new ENM data from toxicology testing to derive OEBs is shown in Figure 7-2.

NIOSH is evaluating the Army ENMs within this same framework. A key advantage of this approach is linking the available data and information sources to leverage existing information for comparison of toxicity to ENMs. The complete linkage scheme is illustrated in Figure 7-3. Note that this structure facilitates comparative toxicity analyses through a "parallelogram" approach [Sobels 1977; NRC 1987; Sutter 1995].

The potential use of ATS data (e.g., in a tiered testing scheme) in the derivation of OELs/OEBs or environmental exposure limits has been discussed [Crump et al. 2010; Maier 2011; Kuempel et al. 2012; Nel et al. 2013]. The evidence basis and possible methods for developing OELs or OEBs based on ATS data, as well as challenges and uncertainties, are discussed below. For example, in addition to the obtaining NOAEL or benchmark dose (BMD) estimates for comparative analyses of ENMs and benchmark materials, the extrapolation of those estimates to humans is a key area of uncertainty that impacts the utility of the experimental data.

Example of Carbon Nanotubes

In a quantitative risk assessment (QRA) of occupational exposure to carbon nanotubes or nanofibers (CNT or CNF), NIOSH used ATS data in the form of *in vivo* short-term dose-response data in rats or mice exposed to various types of CNT or CNF by inhalation, pharyngeal aspiration, or intratracheal instillation, to supplement the subchronic inhalation data in rats for two types of CNTs. The critical dose estimates based on these various types of *in vivo* studies were relatively low, as were the estimated human-equivalent 45-yr working lifetime exposure (average airborne mass concentration 8-hr/d), associated with early-stage adverse lung effects of pulmonary inflammation and fibrosis in rodents [NIOSH 2013a].

Other risk assessments for CNT resulted in a range of proposed OELs from 1 to 50 μg/m^3 (Table 7-1). All of these risk assessments utilized some subset of the same published toxicology studies in rodents, and either the same or similar PODs (i.e., NOAEL or LOAEL) as reported in the studies or BMD estimated from the dose-response data [NIOSH 2013a]. The main reason for the difference in the proposed OELs are the methods and assumptions used to extrapolate from animal dose to humans. Despite the 50-fold range in the OEL estimates for CNT and CNF, all of these OELs are relatively low compared to the regulatory OELs for other types of carbon particles (e.g., carbon black, graphite- natural or synthetic), which range from 2,400 – 5,000 μg/m^3 (2.4 to 5 mg/m^3) of respirable dust (i.e., airborne particle sizes capable of depositing in the pulmonary region of the respiratory tract). The variability in the proposed CNT OELs may contribute to uncertainty in risk management decision-making (e.g., different engineering controls may be necessary to control exposures to 1-10 vs. 10-100 μg/m^3 concentrations).

Utility of ATS Data in Risk Assessment and OEL/OEB Development

On this backdrop of varied and uncertain risk assessment methods and low mass concentrations of the proposed OELs for CNT and CNF, what is the potential utility, if any, of other ATS data (e.g., *in vitro* data) in reducing the uncertainty and variability in these risk estimates across various types of CNT? For example, is there a benchmark to which the toxicity of other types of CNT could be compared, in order to facilitate and support prevention through design efforts (i.e., to develop safer, less hazardous ENMs)? These questions also apply to other types of ENMs, but CNTs/CNFs provide the opportunity to evaluate the predictability of the ATS data for assessing the relative hazard of this class of ENMs. Recent studies have reported wide differences in the pulmonary inflammatory responses based on surface functionalization, including reduced inflammatory and fibrogenic responses to various types of CNT [Sager et al. 2013; Wang et al. 2012; Li et al. 2013] or to TiO_2 nanospheres and nanobelts [Porter et al. 2013].

Ideally, such *in vitro* data would be used in the relative ranking of the potency of the ENMs within biological MOA categories [Kuempel et al 2012; Nel et al. 2013]. The MOA category to which an ENM is assigned would include a consideration of its physico-chemical properties [Liu et al. 2011, 2013]. The target response endpoints and cell types used in the experimental assays would depend on the MOA (in order to detect the effects of concern). A subset of all ENM tested in the *in vitro* (cell) systems would be selected for the next tier of toxicity testing (short-term *in vivo*

studies in rodents). For example, the lowest, middle, and highest potency substances could be selected for short-term *in vivo* testing, and the correlation between the *in vitro* and *in vivo* dose-response relationships examined. Good concordance of the *in vitro* and *in vivo* findings on the relative potency of a representative subset of ENMs would support inferences of the relative potency in the full set of ENMs in the same MOA category.

Several studies have shown good concordance of the relative hazard of ENMs in *in vitro* and *in vivo* assays of particle-induced inflammation [Donaldson et al. 2008; Rushton et al. 2010; Zhang et al. 2012]. The dose metric in these studies differed, including comparison of either the total particle surface area to the total cell surface area *in vitro* or *in vivo* (cm^2/cm^2) [Donaldson et al. 2008], the response per unit surface area [Rushton et al. 2010], or the area under dose-response curve [Zhang et al. 2012]. Quantitative structure-activity relationships (QSAR) models have been developed to predict the bioactivity of the ENMs based on their physico-chemical properties and the associated response endpoints *in vitro*; for example, QSAR models have been used to classify or cluster metal oxide ENMs into bioactivity groups [Liu et al. 2011, 2013; Rallo et al. 2011]. Although these hazard ranking and clustering analyses do not provide a direct estimate of a POD for use in risk assessment, the *in vitro* results could be used to predict *in vivo* responses through benchmark particles. That is, if each group or cluster of ENMs identified includes benchmark (reference) particles for which *in vivo* PODs have been identified or health-based OELs are available, then initial OELs or OEBs could be derived by linking to the other ENMs in that category.

An apparent threshold for inflammation of the particle surface area per surface area of cells was observed in both *in vitro* and *in vivo* systems (1 cm^2/cm^2) for particles within the poorly-soluble low toxicity (PSLT) category [Donaldson et al. 2008]. This study provides a POD estimate for risk assessment of ENMs within the PSLT category. The use of *in vitro* dose-response data to estimate a POD directly has been proposed, using methods similar to those used for *in vivo* data, including adjustment of the POD by uncertainty factors (initially until more evidence is available) [Crump et al. 2010].

Generally, the *in vitro* assays would be expected to be capable of predicting only the acute *in vivo* responses (e.g., within 24 hr. of exposure). For example, these assays may be useful for highly reactive substances. However, several recent studies have shown correlation between the activation of the NLRP3 inflammasome and pro-fibrogenic endpoints *in vitro* or fibrosis *in vivo*

associated with exposure to CNT [Wang et al. 2011, 2012; Li et al. 2013; Sager et al. 2013; Hamilton et al. 2013]. Thus, with further validation, an *in vitro* inflammasome activation assay may be useful for assessing the potential for chronic adverse effects of CNT and other ENMs.

Dose Measures

Selection of relevant dose metrics and dose levels in experimental studies is essential to characterize the dose-response relationship in the test systems, and to estimate the human-equivalent dose in risk assessment. The choice of dose metric (mass, surface area, volume, number) may influence the dose-response relationships, but may need to be converted back to mass concentration in order to align with standard exposure measurement methods in the workplace (e.g., for ultrafine TiO_2 [NIOSH 2011].

A challenge in interpreting results from *in vitro* studies is that the doses are often much higher than occupationally equivalent lung doses. Gangwal et al. [2011] estimate that *in vitro* concentrations of ~0.2-0.6 μg/ml would be equivalent surface area doses to those in humans with a 24-hr exposure to an airborne concentration of 1 mg/m^3 of nanoparticles (TiO_2 and silver). *In vitro* concentrations of 50-68 μg/ml were estimated to be equivalent to long-term (45-year working lifetime) retained human alveolar surface area doses; such concentrations are in the range of typical *in vitro* doses. However, there is considerable uncertainty in the assumption that the acute biological responses *in vitro* would be equivalent to those at an equivalent *in vivo* dose received at a much lower dose rate (i.e., over a 45-year working lifetime) [Oberdörster 2012]. Moreover, these human-equivalent dose estimates were based on an assumed human alveolar surface area of only ~10.6 m^2 [Gangwal et al. 2011]. Alternatively, using the average human total alveolar surface area of ~102 m^2 [Stone et al. 1992], the estimated human-equivalent *in vitro* doses would be lower by an order of magnitude.

Determining the effective dose (i.e., which reaches the target cells) could improve the predictive power of test systems to *in vivo* responses. The particle surface area doses to cells can differ significantly at a given mass concentration (μg/ml) due to the differences in the specific surface area (m^2/g) of particles of different sizes and also to differences in the sedimentation and diffusion properties of particles in liquid-based *in vitro* systems [Hindliter et al. 2010]. The *in vitro* Sedimentation, Diffusion and Dosimetry (ISDD) model was reported to provide improved estimates of dose to cells.

Risk assessment of nanomaterials in animal studies is limited by uncertainty in how well the current animal and human respiratory tract clearance and retention models predict the internal dose of nanomaterials. Human lung dosimetry models have been validated for the long-term retention of respirable poorly soluble particles [Gregoratto et al. 2010]; however, only limited evaluation of these models is available for nanoparticles [Asgharian and Price 2007; MacCalman et al. 2009].

Challenges

A number of challenges remain in the potential use of ATS data in a tiered toxicology testing scheme for OEL/OEB development, including the following:

- Rigorous evaluation and validation of ATS methods is necessary prior to their acceptance for use in QRA and OEL development, including demonstrated reproducibility of assays and high predictability for representative ENMs.
- Currently, the *in vitro* assays may not be sufficiently reproducible for use in either hazard ranking or *in vitro* to *in vivo* extrapolation, due to high variability across laboratories and cell systems [Bonner et al. 2013; Xia et al. 2013].
- Statistical methods to analyze ATS data must be able to accommodate mixed dose-response relationships and account for variability and heterogeneity in the data from multiple response endpoints, cellular assays, and other experimental conditions.
- Standardized risk assessment methods will be needed in order to effectively utilize comparative toxicity data (i.e., to impact the OEL/OEB derivation in view of uncertainties in the risk assessment process).

Several nanotoxicology studies provide specific examples of good concordance of the *in vitro* and acute *in vivo* inflammatory and fibrotic responses to carbon and metal ENMs, as discussed above in "Utility of ATS data in risk assessment and OEL/OEB development." However, validation of these findings is needed to support the use of a tiered testing strategy to fill gaps in hazard data needed to derive OELs or OEBs. Such validation could be achieved through analyses including benchmark or reference particles.

Coordination of tiered toxicology testing methods and data analyses for hazard and risk assessment

To streamline the use of toxicology research in risk assessment and risk management decision-making, the experimental studies should be designed in close collaboration between the experimental toxicologists and the computational toxicologists, statisticians, and risk assessors. Since a key outcome from the experimental studies for use in risk assessment is the determination of an effect level or POD, experiments should be designed with sufficient dose groups to characterize the dose-response relationship in the low dose region (including at occupationally relevant doses.

Benchmark dose modeling of the dose-response relationship can be used to estimate the dose associated with an adverse response. BMD estimates have several statistical advantages over no NOAELs for use in risk assessment [Crump 1984, 1995, 2002; US EPA 2010]. Thus, BMD estimation should be a standard part of the data analysis for ENMs evaluated within the sets of standard assays. At a minimum, the BMD analyses would simply involve the use of standard BMD modeling software [US EPA 2012]. Any data that are insufficient to be adequately fit in BMDs would be identified for possible level two analysis. The set of toxicology assays would be designed to facilitate BMD estimation (e.g., *in vitro*, 4-5 dose groups, n=10 reps; including occupationally relevant doses). These tier I *in vitro* assays would include those shown to be predictive of in vivo responses, focusing on the lungs (e.g., ROS generation and acute inflammation, inflammasome activation and fibrosis, long-term *in vitro* genotoxicity assays) but also with the addition of other cells or target organs if MOA data suggest relevance to workers. The limited tier II *in vivo* assays would be performed (or existing data would be utilized) for benchmark particles in the MOA categories. A future effort to evaluate an initial set of data would also provide an opportunity for additional evaluation and validation or refinement of the initial set of assays/analyses.

Obtaining this standard set of PODs/endpoints would facilitate the use of the tiered toxicology assay data in grouping and ranking ENMs for hazard and risk assessment. Moreover, setting up a standard data file structure for entering the experimental data needed for dose-response modeling of key endpoints and predictive factors would facilitate their use for comparative toxicity and potency analyses as well as QSAR analyses. If a standard set of benchmark assays and

endpoints were adopted more widely, it would also facilitate the grouping/sharing of data for analyses and reduce laboratory and method variability [Bonner et al. 2013; Xia et al. 2013].

Comparison of Hazard/Control Banding Results with Associated OEBs and OELs for ENMs

The ENMs evaluated in Task 5 control banding are shown in Table 7-3, which provides a comparison of the control banding results, the associated OEBs (Figure 7-2), and the current OELs for the nanoparticles, if available, or for respirable particles with the same chemical composition. This evaluation provides insights into the utility of the current ENM hazard/control banding tools and on the level of exposure control based on banding compared to that indicated by the OEL.

Several findings are apparent in Table 7-3:

First, the derived ENM control bands are either "RL3: Containment" or "RL4: Seek specialist advice." The associated OEB for "RL3: Containment" is 1-10 μg/m^3 (Figure 7-2). Although there is no OEB for "RL4: Seek specialist advice," a prudent OEB (in the absence of other information) is suggested as <1 μg/m^3 (Closed Systems and Robotics). As shown in Task 5 (and Appendix A), the driving factor in identifying the appropriate exposure control measure using control bandings is availability of relevant information.

Second, for the poorly-soluble particle groups, general consistency is observed between the control band/OEB and relative hazard category (lower or higher toxicity), given the two control banding options; some consistency is also observed among the other MOA categories, although further refinement may be possible with additional data.

Third, most of the OELs are considerably higher than the OEBs. Only CNT, silver, and cadmium selenide quantum dots (Cd PEL) have roughly similar OELs to the OEBs.

Finally, considerable variability is observed among the OELs that have been proposed for these nanoparticles (see Table 7-1), yet all are much lower than the OELs for the bulk material.

Further analyses of selected benchmark particles will provide a basis for developing risk-based categorical OELs for the nanoparticles within MOA and PC categories. An example of hazard/risk ranking is shown in Table 7-4 for the poorly-soluble particles categories (low to high toxicity),

including both fine (microscale) and ultrafine (nanoscale) respirable particles. These particles were studied in chronic inhalation studies in rats (including NTP bioassays). The response is lung cancer, and the estimated retained lung dose associated with 1/1,000 excess risk in rats was extrapolated to humans (8-hr TWA concentration, 45-year working lifetime). Those TWA concentrations were aligned with the OEBs (shown in Figure 7-2) to derive the hazard ranking and OEB designations shown in Table 7-4. These results have shown that chemical composition (across particle sizes) was more important than the particle size on the hazard ranking. The ultrafine particles evaluated in these bioassays are assigned a Moderate hazard ranking (OEB 100-1,000 μg/m^3), which is consistent with the NIOSH REL for titanium dioxide nanoparticles (300 μg/m^3) (Table 7-3). The only chemical with both ultrafine and fine particle sizes for comparison is titanium dioxide. Fine TiO_2 was assigned a low hazard rank (>1,000 μg/m^3), which is also consistent with the NIOSH REL for fine titanium dioxide (2,400 μg/m^3). The reason for this consistency is that these NIOSH RELs are based on the same criteria (<1/1000 excess risk) [NIOSH 2011]. [Note: these excess risk estimates are the 95% lower confidence interval estimates].

Another material included in both Tables 7-3 and 7-4 is nickel. In this case, the risk-based OEBs (1-10 and 10-100 μg/m^3, for nickel subsulfide and nickel oxide, respectively) (Table 7- 4) are fairly consistent with the NIOSH REL (15 μg/m^3), which also has a carcinogen classification. The control band (RL4: Seek specialist advice) and associated (suggested) OEB of 1 μg/m^3 also indicate a very high hazard and need for a high level of exposure containment and control.

Some of the ENMs that may warrant high priority for similar quantitative risk-based evaluation include zinc oxide, which has current OELs of 2,000 to 5,000 μg/m^3 (although not for the nanoparticles per se); alumina, which has a current OEL of 5,000 μg/m^3; and graphene, which does not have an OEL (graphite OELs are 2,500 to 5,000 μg/m^3) (Table 7-3).

Next Steps

Among the ENMs in Table 7-3, and the Army ENMs in the TEARR database (Task 1) with sufficient data, further risk-based analyses and control banding can be performed for specific exposure scenarios of interest to the Army. These findings will aid in the development and application of categorical OELs/OEBs for ENMs based on physico-chemical properties and biological MOA.

These findings will also be relevant to the tiered toxicological testing scheme (Task 2). That is, these benchmark materials within the MOA categories will be included in the same experimental assays (as reference materials or controls) with the ENMs to facilitate the comparison of toxicity and potency, and the assignment of an initial OEB for new ENMs based on tier 1 and limited tier II testing. The comparative toxicity linkages will be feasible because many of the benchmark materials already have tier III testing data (e.g., the particles in Table 7-4).

The TEARR database [Money et al. 2013] will be useful in these evaluations, especially if it can be enhanced with more detailed and specific toxicology data, including NOAELs or LOAELs, as well as information on bio-solubility and other PC properties than can be used in QSAR modeling. These data, along with data on the benchmark or reference materials along with ENMs in the tiered toxicology assays, could also be useful for future QSAR modeling. A key objective of this work is to develop the database needed for QSAR and other predictive toxicity modeling.

Pulmonary responses to ENMs is a focus area of ongoing NIOSH studies to evaluate the potential hazard of airborne nanomaterials in the workplace. As part of the data analyses to examine the dose-response relationships, compare potency, and develop OELs or OEBs for ENMs, NIOSH is developing databases to evaluate *in vivo* lung responses to micro- and nano-diameter particles. Key variables for these data analyses include the following (Note: the applicable units for each parameter must be recorded as well):

Study exposure and animal information

- Chemical name
- Study reference citation
- Species
- Gender
- Number of animals per group
- Route of exposure
- Administered dose(s) or Airborne concentration(s)
- Duration of exposure (hr/d, d/wk, wks)
- Duration of post-exposure
- Lung dose measured (deposited and end of exposure)

- Body weight (start and end of exposure)
- Lung weight (start and end of exposure)

Particle properties

- Primary particle diameter (mean/median and SD/GSD)
- Agglomerated particle diameter (mean/median and SD/GSD)
- Airborne particle diameter (e.g., MMAD and GSD)
- Specific surface area (m^2/g)
- Solubility
- Surface Reactivity

Key Pulmonary Responses

- Bronchoalveolar lavage (BAL)
- Total cell number (and cell number counted for differentials)
- PMN (count and percent of total)
- Alveolar macrophages (count and percent of total)
- Lactate deyhrogenase (LDH)
- Albumin
- Fibrosis category (distribution and severity)
- Tumor proportion

In future literature searching and data mining efforts, NIOSH suggests including this information, at a minimum, to facilitate the extension of the *in vivo* data base and comparative analyses of pulmonary responses and to serve as points of reference for subsequent evaluations to compare *in vitro* (lung cell) and *in vivo* responses. This list can be extended to include additional other key parameters of particle properties and adverse health endpoints of interest.

Table 7-1. Examples of occupational exposure limits (OELs) proposed for engineered nanomaterials

Nanomaterial	OEL ($\mu g/m^3$)	OELs for substance with same chemical composition ($\mu g/m^3$)*	Reference for Nanomaterial OEL
Titanium dioxide (ultrafine)	610	2,400 – 5,000 (fine, respirable TiO_2)	Gamo 2011; Nakanishi 2011
	300		NIOSH 2011; JSOH 2013
	17		Aschberger et al. 2011
Fullerene (C_{60})	390	2,500 – 5,000 (respirable carbon black or graphite)	Shinohara 2011; Nakanishi 2011
	7.4		Aschberger et al. 2011
MWCNT (Baytubes®)	50	2,500 – 5,000 (respirable carbon black or graphite)	Pauluhn 2010
Carbon nanotubes	30		Nakanishi 2011
MWCNT	1-2		Aschberger et al. 2011; Nanocyl 2009
Carbon nanotubes & nanofibers	1		NIOSH 2013
Silver (nanoparticles)	0.33-0.67	10 – 100 (soluble or insoluble Ag, total dust or fume)	Aschberger et al. 2011

*NIOSH 2007; ACGIH 2012.

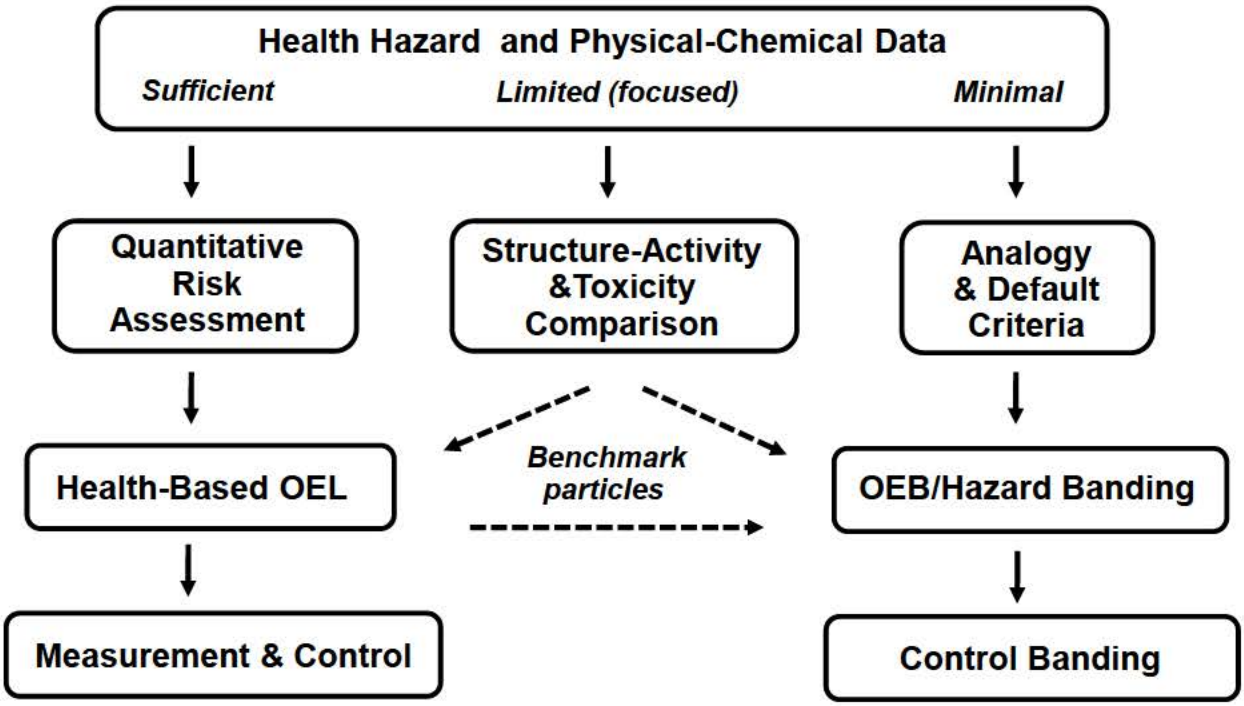

Figure 7-1. Evidence-based strategy to develop exposure control limits and bands for engineered nanomaterials (ENMs), based on level of evidence. Abbreviations: Occupational exposure limit (OEL); Occupational exposure band (OEB). [Adapted from Kuempel et al. 2007, 2012; Schulte et al. 2010].

Table 7-2. Data and methods needed to develop occupational exposure limits or bands (OELs or OEBs)

Guidance value*	Level of Evidence	Data, Analysis Tools and Methods
Individual OEL	Sufficient	Individual (substance-specific) dose-response data for quantitative risk assessment; availability of substance-specific sampling and analytical method
Categorical OEL or OEB	Limited (focused)	Comparative toxicity, clustering & categorization to estimate hazard or risk based on physical-chemical properties and biological mode-of-action data
OEB	Minimal or inadequate	Analogy; default hazard categories and exposure control options are applied.

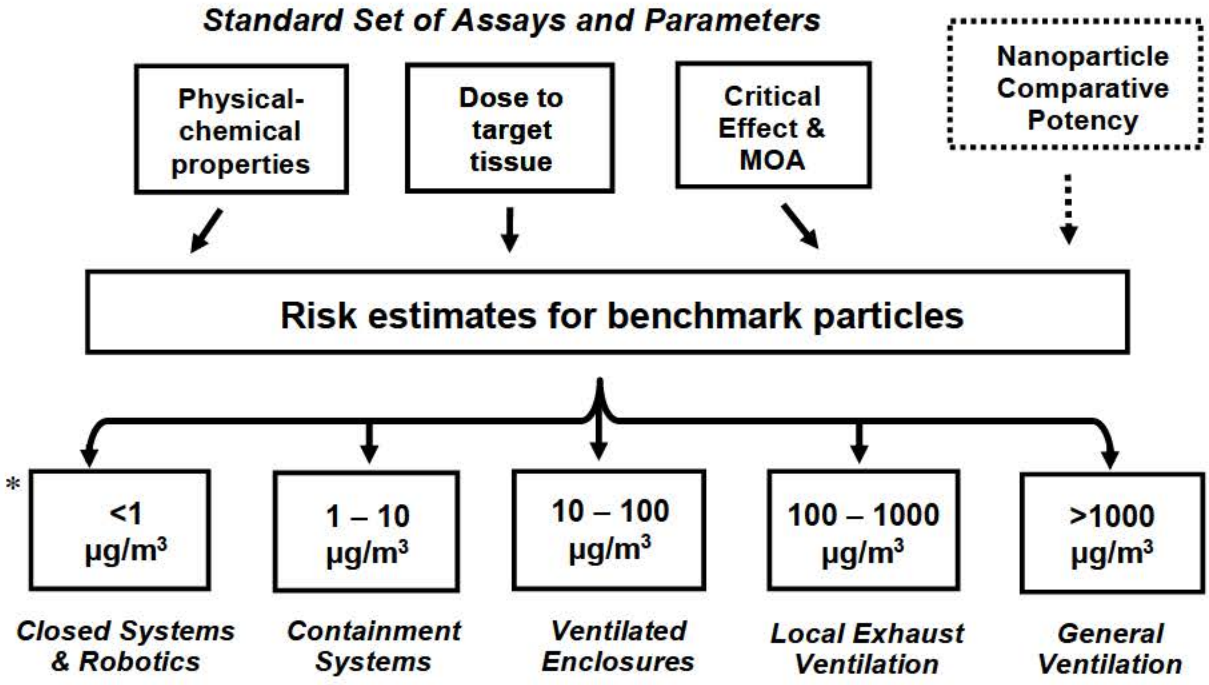

Figure 7-2. Implementing risk assessment into hazard and exposure control banding – an example of order of magnitude bins.*

* 8-hr time-weighted average concentration. Exposure control limit bands and engineering control systems based on: Naumann et al. [1996]; Ader et al. [2005]; Zaik and Nelson [2008]. [Source: Kuempel et al. 2012]

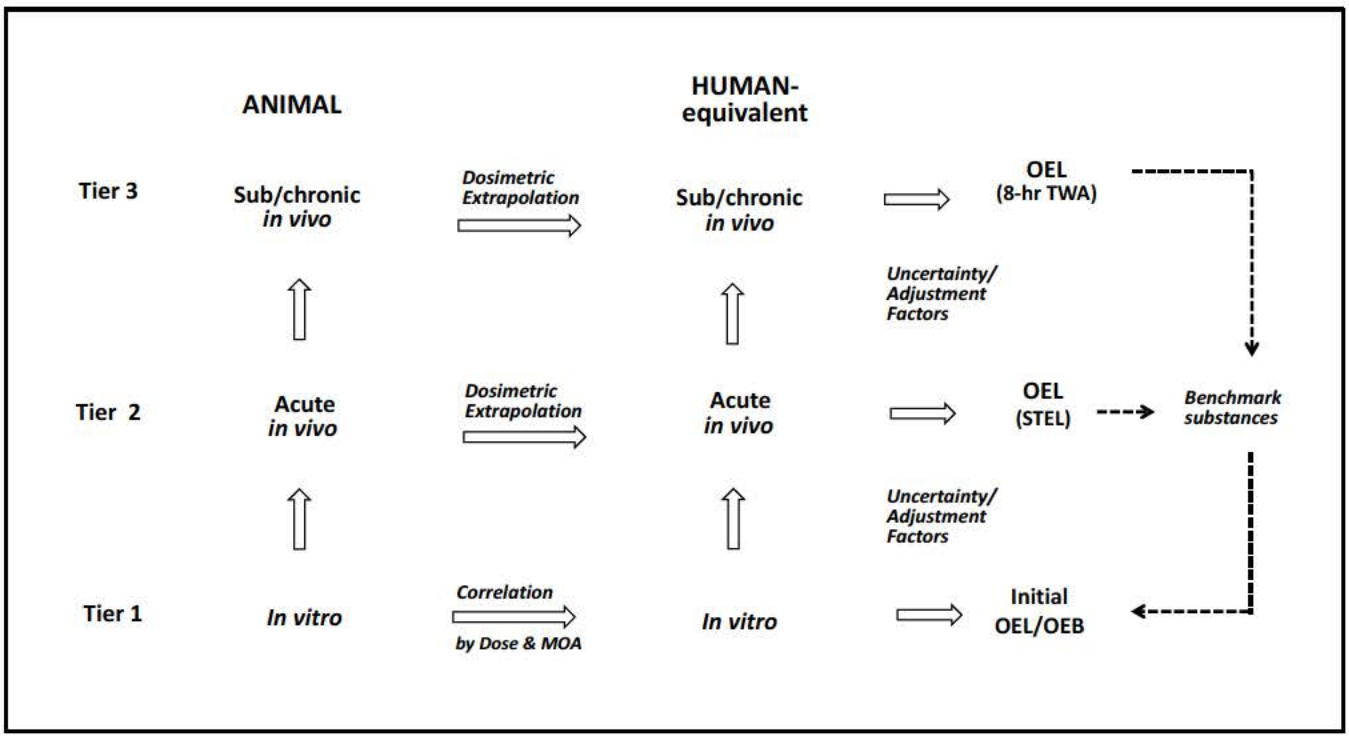

Figure 7-3. Tiered Toxicology Testing Framework for Developing Occupational Exposure Limits or Bands for ENMs.

[Based on: Kuempel 2013, 2014. Other related frameworks include: Sobels 1977; NRC 1987; Sutter 1995; Oberdörster et al 2005; Maier 2011; Cotes et al 2012].

Table 7-3. Evaluation of control bands, associated OEBs, and OELs for ENMs evaluated in Task 5.

Initial MOA Category	ENM	TEARR Category (& No.)	Control Band (Task 5)	OEB Figure 8-2, (µg/m^3), 8-h TWA	OEL (µg/m^3), 8-h TWA
Fibrous (high aspect ratio) particles	CNT (SWCNT, MWCNT, CNF)	Carbon-based (5, 10)	RL4: Seek Specialist Advice	(<1)	1 – 50* 1 (REL)
Higher solubility particles – acute toxicity subcategory	Nickel NP	Metals (23)	RL4: Seek Specialist Advice	(<1)	15 (REL) – Ca 1,000 (PEL)
	Zinc Oxide (ZnO)	Metal oxides (35)	RL3: Containment	1-10	5,000 (REL, PEL) 2,000 (TLV)
Higher solubility particles – lower acute toxicity subcategory	Alumina (Al_2O_3)	Metal oxides (28)	RL3: Containment	1-10	5,000 (PEL)
	Silver NP	Metals (14)	RL3: Containment	1-10	10 sol; 100 insol (TLV) 10 (REL, PEL) 0.1-0.67*
Poorly-soluble, low toxicity (PSLT) particles	Titanium dioxide (TiO_2) NP	Metal oxides (34)	RL3: Containment	1-10	17 – 610* 300 (REL)
	Tungsten nanoparticles (W)	Inorganics (37)	RL3: Containment	1-10	1,000 sol; 5,000 insol (REL & TLV)
Poorly-soluble, high toxicity (PSHT) particles	Silica (SiO_2)	Metal oxides (33)	RL4: Seek Specialist Advice	(<1)	50 (REL) 100 (PEL)
	Cadmium selenide quantum dots (CdSe)	Quantum dots (41)	RL4: Seek Specialist Advice	(<1)	5 (Cd, PEL) - Ca
	Graphene	Carbon-based (7)	RL4: Seek Specialist Advice	(<1)	None 2,500-5,000 (Graphite REL, PEL)

*Table 7-1.

Table 7-4. Example Benchmark Particles & Risk-based Occupational Exposure Bands: Poorly-Soluble Inhaled Particles

Hazard Rank	Substance	Primary Particle Size	Occupational Exposure Band* (8-hr TWA, µg/m^3)
Low	Molybdenum oxide Titanium dioxide (F)	Fine	>1,000
Moderate	Carbon black Diesel exhaust particulate Titanium dioxide (UF)	**Ultrafine**	100 – 1,000
High	Nickel oxide	Fine	10 – 100
Very high	Nickel subsulfide Gallium arsenide	Fine	1–10

*Assignment based on working lifetime exposures associated with <1/1000 excess risk of lung cancer; 95% LCL estimates extrapolated from rat chronic inhalation studies by NTP.

Source: based on results in Kuempel et al. [2012].

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Appendix A. Control Banding Evaluations for Selected Nanomaterials

Control Banding Nanotool evaluation - Alumina

Hazard severity determination descriptors	Severity Score	Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10	All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	0	Al_2O_3 is compact or spherical
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5	Assuming 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7.5 points	10	Insoluble
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently Unknown
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	4.5	Currently unknown
Toxicity: OEL of parent material <10 µg/m^3 =10 points 10-100 µg/m^3 = 5 points 101 µg/m^3 to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	0* ***Based on Al_2O_3**	NIOSH REL for Al_2O_3, 10 mg/m^3 (total dust) 5 mg/m^3 (respirable dust)
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Al_2O_3 alone is not considered to be carcinogenic
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Al_2O_3 alone is not considered a dermal hazard
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Al_2O_3 alone is not considered a asthmagen
Total Severity Score	**47.5**	**MEDIUM**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL 3 - Containment

Control Banding Nanotool evaluation – Silver NPs

Hazard severity determination descriptors	Severity Score	Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10	All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	0	NMs assumed to be spherical
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5	Assuming 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7.5 points	10	Insoluble in H_2O
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	3	Currently unknown
Toxicity: OEL of parent material <10 $\mu g/m^3$ =10 points 10-100 $\mu g/m^3$ = 5 points 101 $\mu g/m^3$ to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	0* ***Based on Silver**	Silver NIOSH REL 0.01 mg/m^3
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Silver alone is not considered to be carcinogenic
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Silver alone is not considered a dermal hazard
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Silver alone is not considered a asthmagen
Total Severity Score	**46**	**MEDIUM**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL3 – Containment

Control Banding Nanotool evaluation - Graphene

Hazard severity determination descriptors	Severity Score	Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10	All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	10	Flat. Un-rolled tube or flattened fiber
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5	Assumed 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7.5 points	10	Insoluble
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	3	Currently unknown
Toxicity: OEL of parent material <10 $\mu g/m^3$ =10 points 10-100 $\mu g/m^3$ = 5 points 101 $\mu g/m^3$ to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	0* ***Based on Carbon**	Carbon NIOSH REL 2.5 mg/m^3
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Carbon alone is not considered to be carcinogenic
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Carbon alone is not considered a dermal hazard
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Carbon alone is not considered a asthmagen
Total Severity Score	**56.0**	**HIGH**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL4 – Seek Specialist Advice

Control Banding Nanotool evaluation – Cadmium Selenide QDs

Hazard severity determination descriptors	Severity Score		Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10		All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	0		Cadmium selenide is compact or spherical
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5		Assuming 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7.5 points	10		Insoluble
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	6		Yes, Cadmium selenide
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5		Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5		Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5		Currently unknown
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	3		Currently unknown
Toxicity: OEL of parent material <10 µg/m^3 =10 points 10-100 µg/m^3 = 5 points 101 µg/m^3 to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	10* ***Based on Cd**		NIOSH REL lowest feasible OSHA PEL 0.005 mg/m^3
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	4*		Cd is considered to be carcinogenic
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*		Cd alone is not considered a dermal hazard
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*		Cd alone is not considered a asthmagen
Total Severity Score	**61.5**		**HIGH**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL-4 Seek Specialist Advice

Control Banding Nanotool evaluation – Tungsten NPs

Hazard severity determination descriptors	Severity Score	Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10	All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	0	Tungsten is compact or spherical
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5	Assuming 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7.5 points	10	Insoluble
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4 5 points	4.5	Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	3	Currently unknown
Toxicity: OEL of parent material <10 µg/m^3 =10 points 10-100 µg/m^3 = 5 points 101 µg/m^3 to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	0* ***Based on Tungsten**	NIOSH REL 5 mg/m^3 TWA
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Tungsten alone is not considered to be carcinogenic
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Tungsten alone is not considered a dermal hazard
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Tungsten alone is not considered a asthmagen
Total Severity Score	**46.0**	**MEDIUM**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL 3 - Containment

Control Banding Nanotool evaluation – Titanium dioxide

Hazard severity determination descriptors	Severity Score	Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10	All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	0	TiO_2 is compact or spherical
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5	Assuming 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7.5 points	10	Insoluble
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	6	Yes, TiO_2 is carcinogenic
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	3	Currently unknown
Toxicity: OEL of parent material <10 $\mu g/m^3$ =10 points 10-100 $\mu g/m^3$ = 5 points 101 $\mu g/m^3$ to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	2.5*	NIOSH REL for fine TiO_2 2.4 mg/m^3
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	4*	Yes, TiO_2 is carcinogenic
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Silver alone is not considered a dermal hazard
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Silver alone is not considered a asthmagen
Total Severity Score	**50**	**MEDIUM**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL3 - Containment

Control Banding Nanotool evaluation – Nickel NPs

Hazard severity determination descriptors	Severity Score	Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10	All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	0	Compact or spherical
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5	Assuming 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7 5 points	10	Insoluble in H_20
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	6	Considered to be carcinogenic
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	6	Considered to be dermally toxic
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	3	Currently unknown
Toxicity: OEL of parent material <10 µg/m^3 =10 points 10-100 µg/m^3 = 5 points 101 µg/m^3 to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	2.5* ***Based on nickel**	NIOSH REL 0.015 mg/m^3
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	4*	Nickel considered to be carcinogenic
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	4*	Nickel considered a skin sensitizer
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Nickel alone is not considered a asthmagen
Total Severity Score	**59.5**	**HIGH**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL4 – Seek Specialist Advice

Control Banding Nanotool evaluation – Zinc oxide

Hazard severity determination descriptors	Severity Score	Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10	All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	0	ZnO is compact or spherical
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5	Assuming 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7.5 points	10	Insoluble in H_2O
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	3	Currently unknown
Toxicity: OEL of parent material <10 µg/m^3 =10 points 10-100 µg/m^3 = 5 points 101 µg/m^3 to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	0 ***Based on ZnO**	NIOSH REL dust 5 mg/m^3
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	ZnO is not considered to be carcinogenic
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	ZnO is not considered a dermal hazard
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	ZnO alone is not considered a asthmagen
Total Severity Score	**46**	**MEDIUM**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL 3 – Containment

Control Banding Nanotool evaluation – Silica oxides

Hazard severity determination descriptors	Severity Score	Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10	All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	0	Silica oxides are compact or spherical
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5	Assuming 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7.5 points	10	Insoluble
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	6	Yes, silica oxides are carcinogenic
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	3	Currently unknown
Toxicity: OEL of parent material <10 µg/m^3 =10 points 10-100 µg/m^3 = 5 points 101 µg/m^3 to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	0* ***Based on SiO_2**	NIOSH REL for Silica 6 mg/m^3
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	4*	Crystalline silica has been linked to silicosis
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Silica alone is not considered a dermal hazard
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Silica alone is not considered a asthmagen
Total Severity Score	**51.5**	**HIGH**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL 4 – Seek specialist advice

Control Banding Nanotool evaluation - CNT

Hazard severity determination descriptors	Severity Score	Supporting Evidence
Surface chemistry High surface reactivity = 10 points Medium surface reactivity = 5 points Low surface reactivity = 0 points Unknown = 7.5 points	10	All NMs have the potential for increased surface reactivity
Particle shape Tubular or fibrous = 10 points Anisotropic = 5 points Compact or spherical = 0 points Unknown = 7.5 points	10	CNTs are in the form of tubes
Particle diameter 1-10 nm = 10 points 11-40 nm = 5 points 41-100 = 0 points Unknown = 7.5 points	5	Assuming 40 nm width
Solubility Insoluble = 10 points Soluble = 5 points Unknown = 7.5 points	10	Assuming insoluble
Carcinogenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Only when combined with a promoter. Currently unknown
Reproductive toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Mutagenicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Dermal toxicity Yes = 6 points No = 0 points Unknown = 4.5 points	4.5	Currently unknown
Asthmagen Yes = 4 points No = 0 points Unknown = 3 points	3	Currently unknown
Toxicity: OEL of parent material <10 $\mu g/m^3$ =10 points 10-100 $\mu g/m^3$ = 5 points 101 $\mu g/m^3$ to 1 mg/m^3 =2.5 points >1mg/m^3 = 0 points Unknown = 7.5 points	0* ***Based on Carbon**	Carbon NIOSH REL 2.5 mg/m^3
Carcinogenicity of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Carbon alone is not considered to be carcinogenic
Dermal hazard of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Carbon alone is not considered a dermal hazard
Asthmagen potential of parent material Yes = 4 points No = 0 points Unknown = 3 points	0*	Carbon alone is not considered a asthmagen
Total Severity Score	**56.0**	**HIGH**

Exposure probability determination descriptors for	Probability Score	Supporting Evidence
Dustiness/mistiness High = 30 points Medium =15 points Low = 7.5 points None = 0 points Unknown = 22.5 points	30	Assumed to be handled in powder form.
Estimated amount of nanomaterial used >100 mg = 25 points 11-100 mg = 12.5 points 0-10 mg = 6.25 Unknown = 18.75	18.75	Unknown amount handled
Number of employees with similar exposure > 15 employees = 15 points 11-15 employees = 10 points 6-10 employees = 5 points 1-5 employees = 0 points Unknown = 11.25 points	0	Assume 1-5 employees
Frequency of operation Daily = 15 points Weekly = 10 points Monthly = 5 points < monthly = 0 points Unknown = 11.25 points	15	Assume daily handling
Duration of operation > 4 hours = 15 points 1-4 hours = 10 points 30-60 minutes = 5 points < 30 minutes = 0 points Unknown = 11.25 points	15	Assume 4-6 hours
Total exposure probability score	**78.75**	**PROBABLE**

RL4 – Seek Specialist Advice